

CHAPTER 16

IMMUNOLOGIC ASSESSMENT

INTRODUCTION

Background

Of the many chemical compounds known to cause immune system dysfunction in laboratory animals, the polyhalogenated aromatic hydrocarbons have been the most extensively studied and, among these, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has proven to be the most toxic. Since TCDD-induced immunotoxicity was first reported in experimental animals in the early 1970's (1, 2, 3), a large body of literature pertinent to this subject has accumulated and has been summarized in previous reports from the Air Force Health Study (AFHS) (4, 5).

In laboratory animals, numerous studies have demonstrated that TCDD has a wide range of toxic effects and is a potent suppressor of both humoral- and cell-mediated immune function (6, 7). In mice, TCDD has been shown to cause myelosuppression (8), impaired lymphoproliferative responses and humoral antibody production (9, 10), thymic atrophy (11), and impaired complement activity (12). More recent research has focused on defining the mechanisms of TCDD-induced immune dysfunction. Some, but not all of the manifestations of TCDD toxicity are clearly related to the presence of the aryl hydroxylase (Ah) receptor that is present in lymphoid tissue and lymphoid cells (13-16). Myelotoxic effects (17, 18), suppression of humoral antibody responses (19), and impaired complement activity (20) are among those that have been proven to be Ah receptor mediated. In contrast, numerous investigators have established that the effects of TCDD on B-cell maturation can occur independent of the presence of the Ah receptor (21, 22, 23). In one study, the primary target for TCDD-induced suppression of IgM antibody production was found to be the B lymphocyte at the level of cell differentiation (24). Further, there is good evidence that the age of the experimental animal is an important determinant of several immune system consequences of dioxin (25), including the responsiveness of thymocytes to Interleukin 1 (26), and the more persistent thymic atrophy and suppression of cell-mediated immunity seen in perinatal versus adult mice (27).

It is difficult to extrapolate the results of these animal studies to humans for a number of reasons. Doses of TCDD administered were extreme by any measure of human dioxin exposure, routes of administration were usually not comparable, interspecies variation is unpredictable, and the period of observation was insufficient to reflect the latency effect that may be required to produce clinical endpoints in humans. As noted in Chapter 15, Endocrine Assessment, there are similarities in the physicochemical properties of the Ah receptor in animals and those that mediate the effects of thyroid and glucocorticoid hormone function in humans. To date, a receptor capable of binding TCDD has been defined in several human tissues (including placenta [28], skin [29], and lung [30]), and an Ah receptor has been identified in cultured human thymic epithelial cells. Initial characterization of its physicochemical properties has been the subject of several reports (31, 32).

In contrast to the active research in animals, relatively few studies have been published describing immune system effects of TCDD in humans and, from these, no consistent evidence for immunologic abnormalities has emerged. An apparent impairment in cell-mediated immunity was found after an environmental exposure (33) but was not confirmed in followup observations (34). A more recent report examining immunologic indices and, for the first time, correlating the results with the body burden of dioxin based on adipose tissue levels, found no evidence for any immune system impairment (35). These findings are consistent with those recently reported in the AFHS (5).

Earlier studies of the effects of TCDD on the human immune system have been limited by unreliable indices of dioxin exposure and/or insufficient followup to reflect a latency effect. Though the severe consequences of advanced immune suppression in humans (overwhelming infection and malignancy) are well established, reliable clinical and laboratory indices to detect more subtle compromise in immune function are not well understood. In this regard, two recent publications have made valuable contributions to consistency in laboratory methodology and quality control (36, 37).

More detailed summaries of the pertinent scientific literature for the immunologic assessment can be found in the report of the previous analyses of the 1987 examination data (5).

Summary of Previous Analyses of the 1987 Examination Data

For the assessment of the 1987 immunologic examination data, composite skin reaction test results and various laboratory examination measurements from cell surface marker studies, three groups of functional stimulation tests, and quantitative immunoglobulins were analyzed. Ranch Hands had a higher frequency of individuals with possibly abnormal reactions on skin testing than the Comparisons. The analysis of the composite skin test results, adjusting for covariate information, contained a significant group-by-lifetime cigarette smoking history interaction. Followup analyses showed that, among those individuals with the heaviest smoking histories, Ranch Hands had a higher frequency of possibly abnormal readings when contrasted with Comparisons. Within the other strata, there were no significant differences. The unadjusted analyses of the laboratory examination data indicated no significant group difference between Ranch Hands and Comparisons. For the adjusted analyses of the natural killer assay measurements with and without Interleukin 2 (IL-2), significant interactions between group and race were present. Exploration of these interactions revealed that the Black Ranch Hands had higher adjusted means than the Black Comparisons for the natural killer assay measures. The adjusted mean values for Black Ranch Hands, non-Black Comparisons, and non-Black Ranch Hands were numerically similar in these analyses. Black Comparisons had lower mean values than the other three groups. The clinical significance of these findings is not apparent and does not point to any known clinical endpoints. In general, the immunologic assessment revealed no medically important differences between the Ranch Hands and Comparisons.

Parameters of the Immunologic Assessment

Dependent Variables

Data from the physical examination and the Scripps Immunology Reference Laboratory (SIRL) were used in the immunologic assessment. Immunologic tests were carried out on a random sample of approximately 40 percent of the participants because of the complexity of the assay and the expense of these tests. Blood was drawn for testing from approximately one-half of these randomly chosen participants on the first day of the physical examination, and blood was drawn from the rest of the selected participants on the second day.

All participants except those chosen to receive the immunologic tests at SIRL on day 2 of the physical examination were scheduled to receive the skin test as a part of the physical examination (approximately 80 percent of the 1987 examination participants). Participants chosen to receive the immunologic blood draw on day 2 of the physical examination were not given skin tests to avoid any effect the skin test antigens might have on the cell counts and functions.

Physical Examination Data

Physical examination data concerning the skin tests were used to evaluate immunologic function. A composite skin test diagnosis variable was constructed based on the response to four separate antigens injected interdermally to measure antigen reactivity or sensitivity. This composite skin test variable was analyzed as a discrete, dichotomous variable: each participant was considered possibly abnormal or normal based on his skin reactivity to the antigens *Candida albicans*, mumps, Trichophyton, and staphage-lysate. The response to each antigen was scored positive (normal) if the maximum diameter of the resulting 48-hour induration was greater than or equal to 5 mm, which indicated intact cell-mediated immunity. If none of the four antigen responses was positive, the composite skin test diagnosis was scored possibly abnormal. If one or more of the four antigen responses was positive, the composite skin test was considered normal.

Participants taking anti-inflammatory (except aspirin) or immunosuppressant medication, or who had recently received x-ray treatment or chemotherapy for cancer (as reported in the 1987 health interview questionnaire and verified by medical records review) were excluded from all analyses of skin test data. In addition, data from participants in examination group 2, except for one participant, were not used in the analysis of the composite skin test diagnosis variable, since they received staphage-lysate at a different dosage than all the other examination groups. One of the two nurses made a dosage error affecting all but the one participant in examination group 2.

Laboratory Examination Data

From the SIRL immunologic tests, the results of cell surface marker studies, total lymphocyte count (TLC), functional stimulation studies, and quantitative immunoglobulins were analyzed. Figure 16-1 presents the immunologic parameters evaluated and describes their medical importance. In the report on the 1987 examination, these data were evaluated to determine whether the natural logarithm scale was more appropriate for use with the statistical procedure(s) than the original scale (5). Appendix Table P-1 of the report on the 1987 examination summarized the statistics used in that assessment. The descriptive

FIGURE 16-1.

Medical Significance of the Immunologic Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
Skin Tests		
Candida Mumps Tricophyton Staphage-lysate	Skin testing measures in vivo hypersensitivity responses to antigens of bacteria, fungi, and a virus to which most persons have previously been exposed. The skin reaction to intradermal injection of these antigens indicates integrity of T-cell memory and ability of effector cells to mount a response.	Antigen reactivity or sensitivity. Lack of response to all antigens indicates anergy which may occur in overwhelming infections, widespread malignancy, immunosuppression, or malnutrition.
Marker Studies		
CD2 (T11)	Measures CD2 cells coincident with sheep rosette receptor on cell surface (most are CD4 and CD8 cells). CD2 positive cells represent total T cells.	Decrease may result in cellular immune deficiency; increased with lymphoproliferative disorders.
CD20 (B1)	Measures peripheral blood B cells; no reaction with T cells, granulocytes, or monocytes.	Decrease may result in humoral immune deficiency with impaired production of antibodies; increased in lymphoproliferative disorders.
CD4 (Leu3a+b)	Measures T cells that exhibit helper/inducer phenotype. CD4 cells initiate an immune response to processed antigens.	Markedly decreased in AIDS due to HIV infection of CD4+ cells; increased in autoimmune diseases.
CD8 (OKT8)	Measures T cells that exhibit suppressor/cytotoxic functions. Responsible for appropriate down regulation of an immune response after antigen has been cleared.	Variable in autoimmune diseases; increased in some viral illnesses and immunodeficiencies.

FIGURE 16-1. (Continued)

Medical Significance of the Immunologic Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
CD14 (LeuM3)	Measures mature monocytes in peripheral blood. Monocytes take up and process foreign antigens for presentation to CD4+ cells.	Increases with inflammation of many etiologies.
CD25 (IL-2 Receptor)	Present on activated T cells; absent on normal peripheral blood lymphocytes, monocytes, and granulocytes. Stimulation with IL-2 induces more IL-2 Receptor synthesis in activated T cells (positive feedback).	Increased in lymphoproliferative disorders. Also increased with any immune activation (viral infection, organ transplant rejection).
HLA-DR	Measures cells expressing HLA-DR antigen; includes B cells and monocytes. HLA-DR+ cells present antigen to CD4+ T cells.	Decreased in B-cell deficiency; decreased in agammaglobulinemia. Deficiency may reflect ability to mount primary cellular immune response.
CD4/CD8 Ratio	Measures proportional difference between CD4+ cell populations and CD8+ cell populations. Reflects balance between up regulation and down regulation of T cells.	Decreased in immunodeficiencies and viral illnesses. AIDS causes very low ratio as does immunosuppression with cyclosporine.
TLC	Measures absolute number of total lymphocytes circulating in peripheral blood. Major immune mechanism against fungi and viruses.	Decreased in immunodeficiency; increased in lymphoproliferative disorders.

FIGURE 16-1. (Continued)

Medical Significance of the Immunologic Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
Immunoglobulins		
IgG IgA IgM	Each measures ability of specific B-cell subgroup to secrete specific antibody class of molecules. Antibodies normally rise in response to infections or immunizations with bacteria, fungi, and viruses. Major immune mechanism against bacteria.	Increased in hyperglobulinemia or myeloma (monoclonal). Decreased in selective or total B-cell immunodeficiency. Polyclonal increases in chronic inflammation and liver disease (cirrhosis).
Functional Studies		
PHA	Measures functional capability of T cells to become activated by mitogen and undergo proliferation. Relies on integrity and in vitro interaction of several different cell types including macrophages and T-lymphocytes.	Decreased with impaired natural defenses due to stress, surgery, age, malnutrition, burns, uremia, malignancy, some infections.

FIGURE 16-1. (Continued)

Medical Significance of the Immunologic Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
NKCI (with IL-2) NKCA (without IL-2)	Measures natural killer cell lytic activity with and without Interleukin 2 (IL-2) treatment of the natural killer cells. Percent release relates the amount of chromium-51 released when target cells are killed by natural killer cells to the amount of chromium-51 released when all target cells are killed (maximal release of radioactivity). Net response cpm is generated by the release of isotope from target cells killed by natural killer cells minus the cpm generated by spontaneous lysis or isotope leakage of the target cells. NK activity does not require antibody and is independent of antigen specificity.	Decreased with impaired natural defenses. NK cells are responsible for immunosurveillance in the body. They may attack and destroy virus-infected cells as well as tumor cells arising from carcinogens. NK cells may screen and remove early growths of malignant cells.
MLC	Measures reactivity of T cells to foreign histocompatibility class II antigens on cells from different individuals. Defines HLA-D specificities. Must have several cell types functionally intact as in PHA.	Used for cross-matching HLA-D in organ transplantation. PHA stimulation indicates cellular immune response to very strong mitogen, whereas MLC indicates cellular ability to respond to more subtle antigens on surfaces of living cells. Strong correlation between active PHA and MLC responses.

statistics of skewness and kurtosis were used in conjunction with the Kolmogorov D statistic for deciding whether to use the original scale or the natural logarithm scale (38).

Participants taking anti-inflammatory (except aspirin) or immunosuppressant medication, or who had recently received x-ray treatment or chemotherapy for cancer were excluded from all analyses of laboratory data.

Quantitative Studies: Cell Surface Marker (Phenotypic) Studies

Quantification of the different cell populations was carried out with the use of mouse monoclonal antibodies. Seven cell surface markers and a ratio of cell markers were analyzed in the evaluation of the immunologic system. The unit of measurement (for all variables except the CD4/CD8 ratio) was cells/mm³. These variables were treated as continuous data, and were subjected to the natural logarithm transformation for statistical analysis.

Quantitative Studies: TLC

Statistical analysis on TLC was performed. The unit of measurement was cells/mm³. A natural logarithm transformation was applied to the TLC data for statistical analyses.

Functional Stimulation Tests

Cell function responses to stimulation by phytohemagglutinin (PHA), mixed lymphocyte culture (MLC), and natural killer cell assays were also analyzed in the immunologic evaluation.

The following three PHA variables were analyzed: unstimulated PHA response for 2 mitogen harvest days, an overall PHA net response (adjusting for 3 mitogen concentrations and 2 harvest day effects), and the maximum PHA net response among the 3 mitogen concentrations and 2 harvest days. Each observation was the result of the averaging of quadruplicate readings.

MLC of donor lymphocytes was also used to stimulate in vitro cell proliferation of participant lymphocytes; the following two MLC variables were analyzed: unstimulated MLC response and MLC net response.

The following four variables from the natural killer cell assays were analyzed:

- Natural Killer Cell Assay (NKCA):
 - (1) NKCA 50/1 net response
 - (2) NKCA 50/1 percent release
- Natural Killer Cell Assay with Interleukin 2 (NKCI):
 - (3) NKCI 50/1 net response
 - (4) NKCI 50/1 percent release.

The unit of measurement for the PHA and MLC responses and the natural killer cell assay net response variables was counts per minute (cpm). These variables were treated as continuous in the statistical analysis. A natural logarithm transformation was applied to the unstimulated PHA response and the unstimulated MLC response.

Quantitative Studies: Immunoglobulins

The immunoglobulins IgA, IgG, and IgM were also analyzed statistically. The unit of measurement was mg/dl. The natural logarithm transformation was used in analyses of the immunoglobulins.

Covariates

Covariates used in the immunologic evaluation for adjusted statistical analyses included age, race, current alcohol use (drinks/day), lifetime alcohol history (drink-years), current cigarette smoking (cigarettes/day), and lifetime cigarette smoking history (pack-years). Further, batch-to-batch (examination group) variation and blood draw day-to-day variation (for each examination group) were also used as covariates for laboratory-dependent variables. Study participants who began their physical examination on the same day formed a batch. For the unstimulated PHA response, day of mitogen harvest was also used as a covariate in the adjusted analysis. For the overall PHA net response, mitogen concentration and mitogen harvest day were also used as covariates in the adjusted analyses.

Relation to Baseline, 1985, and 1987 Studies

For the 1985 examination report, the following variables were analyzed for group differences and associations with the exposure index: CD2, CD4, CD8, CD14, CD20, CD4/CD8 ratio, HLA-DR, unstimulated PHA response, PHA net response, MLC net response, and pokeweed net response. All of these variables, except for pokeweed net response, were also analyzed in this report and the previous 1987 examination report. In addition, statistical analyses were also performed in these reports on the following: CD25, unstimulated MLC, TLC, maximum PHA net response, IgA, IgG, IgM, natural killer cell assays with and without Interleukin 2, and the composite skin test diagnosis. Some of the variables in this report were also analyzed in the Baseline study.

Longitudinal analyses were performed on the CD4/CD8 ratio using the data collected for the 1985 and 1987 examinations.

For the 1987 examination report, the PHA net responses were analyzed for each of the six individual combinations of mitogen harvest day and mitogen concentration. In this report, these six analyses were not performed. Instead, the interactions of dioxin-by-harvest day, dioxin-by-mitogen concentration, and dioxin-by-harvest day-by-mitogen concentration were evaluated to determine whether stratified analyses were needed. As a result of those evaluations, the analyses involving initial dioxin in Ranch Hands and categorized current dioxin in the Ranch Hands and Comparisons were performed for each of the three mitogen concentrations.

Statistical Methods

Chapter 4, Statistical Methods, describes most of the basic statistical methods used in the immunologic evaluation. For both the 1985 and 1987 studies, large variation was expected from batch and blood draw day variability. Because of the variation, these covariates were generally incorporated into the unadjusted and the adjusted models of the respective immunologic assessments for those studies. For the serum dioxin analyses of the Ranch Hand immunologic measurements, these covariates were subjected to a prescreening procedure to determine whether the unadjusted and adjusted models would incorporate batch-to-batch and blood draw day-to-day covariates. The prescreening was performed because of the reduced sample sizes available for the stepwise modeling procedure applied to the models involving only the Ranch Hands. In addition, the batch-to-batch and blood draw day-to-day covariates would absorb many of the available degrees of freedom if routinely forced into a particular analysis model.

To address these data issues, a main effects prescreening model with the following terms was used for each immunologic measurement: \log_2 (initial dioxin), batch-to-batch variation, blood draw day-to-day variation, age, race, current alcohol use, lifetime alcohol history, current cigarette smoking, and lifetime cigarette smoking history. The models were used to evaluate the significance of the batch-to-batch and blood draw day-to-day covariates using the data from the maximal cohort (i.e., the larger data set). As a result of that analysis, the batch-to-batch and blood draw day-to-day covariates were used for the unadjusted and adjusted analyses of the following measures: CD14, CD25, HLA-DR, CD4/CD8, unstimulated PHA, PHA net response, maximum PHA net response, unstimulated MLC response, MLC net response, NKCI 50/1 net response, and NKCI 50/1 percent release. The unadjusted and adjusted analyses of CD20 and NKCA 50/1 net response were adjusted only for batch-to-batch variation. Batch-to-batch and blood draw day-to-day variation were not used in the unadjusted and adjusted analyses of CD2, CD4, CD8, TLC, and NKCA 50/1 percent release.

Table 16-1 summarizes the statistical analyses performed for the serum dioxin analyses of the immunologic assessment. The first part of the table describes the dependent variables analyzed. The second part of the table provides a further description of the candidate covariates examined. Abbreviations are used extensively in the body of the table and are defined in footnotes.

Data for four participants (two Ranch Hands and two Comparisons) were judged clinically unreasonable and were excluded prior to analysis. Some participants were excluded from the immunologic evaluation as stated above, and some dependent variable and covariate data were missing for other participants. Table 16-2 summarizes the number of participants excluded for medical reasons and the number of participants with missing data, by assumption and Ranch Hand and Comparison group. Variables used to evaluate skin and immunologic testing are detailed separately in this table, since different subsets of participants received these two types of tests.

Appendix O contains graphic displays of immunology system dependent variables versus initial dioxin for the minimal and maximal Ranch Hand cohorts, and immunology variables versus current dioxin for Ranch Hands and Comparisons. Graphics for dioxin-by-

TABLE 16-1.

Statistical Analysis for the Immunologic Assessment

Dependent Variables

Variable (Units)	Data Source	Data Form	Cutpoints	Candidate Covariates	Statistical Analyses
Composite Skin Test Diagnosis (based on length of four skin test antigen induration measurements)	PE	D	Possibly Abnormal: 0/4 \geq 5 mm Normal: \geq 1/4 \geq 5 mm	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR	U:LR A:LR
CD2 Cells (cells/mm ³)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD4 Cells (cells/mm ³)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD8 Cells (cells/mm ³)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD20 Cells (cells/mm ³)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM

TABLE 16-1. (Continued)

Statistical Analysis for the Immunologic Assessment

Dependent Variables

Variable (Units)	Data Source	Data Form	Cutpoints	Candidate Covariates	Statistical Analyses
CD14 Cells (cells/mm ³)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD25 Cells (cells/mm ³)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
HLA-DR Cells (cells/mm ³)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD4/CD8 Ratio	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM L:GLM
Total Lymphocyte Count (TLC) (cells/mm ³)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM

TABLE 16-1. (Continued)

Statistical Analysis for the Immunologic Assessment

Dependent Variables

Variable (Units)	Data Source	Data Form	Cutpoints	Candidate Covariates	Statistical Analyses
Unstimulated Phytohemagglutinin (PHA) Response (counts/min [cpm])	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH), DAY	U:GLM A:GLM
PHA Net Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH), CONC,DAY	U:GLM A:GLM
Maximum PHA Net Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
Unstimulated Mixed Lymphocyte Culture (MLC) Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
MLC Net Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM

TABLE 16-1. (Continued)

Statistical Analysis for the Immunologic Assessment

Dependent Variables

Variable (Units)	Data Source	Data Form	Cutpoints	Candidate Covariates	Statistical Analyses
Natural Killer Cell Assay (NKCA) 50/1 Net Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
NKCA 50/1 Percent Release	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
Natural Killer Cell Assay With Interleukin (NKCI) 50/1 Net Response (cpm)	LAB	C	--	AGE,RACE CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
NKCI 50/1 Percent Release	LAB	C	--	AGE,RACE CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
IgA (mg/dl)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR	U:GLM A:GLM
IgG (mg/dl)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR	U:GLM A:GLM
IgM (mg/dl)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR	U:GLM A:GLM

TABLE 16-1. (Continued)**Statistical Analysis for the Immunologic Assessment****Covariates**

Variable (Abbreviation)	Data Source	Data Form	Cutpoints
Age (AGE)	MIL	D/C	Born \geq1942 Born <1942
Race (RACE)	MIL	D	Black Non-Black
Current Cigarette Smoking (CSMOK) (cigarettes/day)	Q-SR	D/C	0-Never 0-Former >0-20 >20
Lifetime Cigarette Smoking History (PACKYR) (pack-years)	Q-SR	D/C	0 >0-10 >10
Current Alcohol Use (ALC) (drinks/day)	Q-SR	D/C	0-1 >1-4 >4
Lifetime Alcohol History (DRKYR) (drink-years)	Q-SR	D/C	0 >0-40 >40
Batch-to-Batch (BATCH)	LAB	D	1, 2, 3, ... 80
Blood Draw Day-to-Day (DAY[BATCH])	LAB	D	1, 2 (actual day dependent on batch)
Mitogen Concentration (CONC)	LAB	D	1, 2, 3
Mitogen Harvest Day (DAY)	LAB	D	1, 2

TABLE 16-1. (Continued)
Statistical Analysis for the Immunologic Assessment

Abbreviations

Data Source:	LAB--1987 SIRL laboratory results MIL--Air Force military records PE--1987 SCRF physical examination Q-SR--1987 NORC questionnaire (self-reported)
Data Form:	D--Discrete analysis only C--Continuous analysis only D/C--Appropriate form for analysis (either discrete or continuous)
Statistical Analyses:	U--Unadjusted analyses A--Adjusted analyses L--Longitudinal analyses
Statistical Methods:	GLM--General linear models analysis LR--Logistic regression analysis

TABLE 16-2.**Number of Participants Excluded and With Missing Data for the Immunologic Assessment**

Variable	Variable Use	Assumption		Categorized Current Dioxin	
		(Ranch Hands Only) Minimal	Maximal	Ranch Hand	Comparison
Skin Test Analysis ^a					
Composite Skin Test Diagnosis ^b	DEP	7	12	12	20
Current Alcohol Use	COV	3	5	5	0
Lifetime Alcohol History	COV	6	9	9	1
Chemotherapy	EXC	0	0	0	1
X-Ray Treatment	EXC	1	1	0	2
Anti-Inflammatory or Immunosuppressant Medication	EXC	13	16	13	18
Examination Group 2	EXC	6	6	4	2
Quantitative Immunoglobulins ^c					
Current Alcohol Use	COV	3	5	5	0
Lifetime Alcohol History	COV	6	9	9	2
Chemotherapy	EXC	0	0	0	1
X-Ray Treatment	EXC	1	1	0	2
Anti-Inflammatory or Immunosuppressant Medication	EXC	16	21	19	24

TABLE 16-2. (Continued)
Number of Participants Excluded and With Missing Data for the
Immunologic Assessment

Variable	Variable Use	Assumption		Categorized Current Dioxin	
		(Ranch Hands Only) Minimal	Maximal	Ranch Hand	Comparison
Immunologic Test Analyses ^d					
CD2 Cells	DEP	2	2	3	4
CD4 Cells	DEP	3	3	3	0
CD8 Cells	DEP	3	4	3	0
CD20 Cells	DEP	2	2	2	0
CD25 Cells	DEP	1	1	1	2
HLA-DR Cells	DEP	0	0	0	1
CD4/CD8 Ratio	DEP	4	5	4	0
Unstimulated PHA Response (day 1)	DEP	0	2	2	3
Unstimulated PHA Response (day 2)	DEP	4	5	5	2
PHA Net Response (day 1, conc. 1)	DEP	0	4	4	3
PHA Net Response (day 1, conc. 2)	DEP	0	4	4	3
PHA Net Response (day 1, conc. 3)	DEP	0	4	4	2
PHA Net Response (day 2, all conc.)	DEP	5	6	6	2
Overall PHA Net Response	DEP	5	10	10	4

TABLE 16-2. (Continued)

Number of Participants Excluded and With Missing Data for the Immunologic Assessment

Variable	Variable Use	Assumption (Ranch Hands Only)		Categorized Current Dioxin	
		Minimal	Maximal	Ranch Hand	Comparison
Maximum PHA Net Response	DEP	5	10	10	4
Unstimulated MLC Response	DEP	4	6	7	7
MLC Net Response	DEP	4	6	7	7
NKCA 50/1 Net Response	DEP	6	7	5	11
NKCA 50/1 Percent Release	DEP	6	7	5	11
NKCI 50/1 Net Response	DEP	2	5	5	3
NKCI 50/1 Percent Release	DEP	2	5	5	3
Current Alcohol Use	COV	1	1	1	0
Lifetime Alcohol History	COV	1	1	1	1
Chemotherapy	EXC	0	0	0	1
X-Ray Treatment	EXC	1	1	0	0
Anti-Inflammatory or Immunosuppressant Medication	EXC	6	8	9	9

^aScheduled for 702 Ranch Hands and 664 Comparisons who had a quantified serum dioxin assay.

^bIncludes 31 participants who refused and five equivocal results.

^cPerformed on 866 Ranch Hands and 804 Comparisons who had a quantified serum dioxin assay.

^dPerformed on 324 Ranch Hands and 306 Comparisons who had a quantified serum dioxin assay.

COV--Covariate (missing data).

DEP--Dependent variable (missing data).

EXC--Exclusion.

covariate interactions determined by various statistical models are also presented in Appendix O. Chapter 4 provides a guide to assist in interpreting the graphics.

Three statistical analysis approaches were used to examine the association between an immunology dependent variable and serum dioxin levels. One model related a dependent variable to each Ranch Hand's initial dioxin value (extrapolated from current dioxin values using a first-order pharmacokinetic model). A second model related a dependent variable to each Ranch Hand's current serum dioxin value and each Ranch Hand's time since tour. The phrase "time since tour" is often referred to as "time" in discussions of these results. Both of these models were implemented under the minimal and maximal assumptions (i.e., Ranch Hands with current dioxin above 10 ppt and above 5 ppt, respectively). The third model compared the dependent variable for Ranch Hands having current dioxin values categorized as unknown, low, and high with Comparisons having background levels. The contrast of the entire Ranch Hand group with the complete Comparison group can be found in the previous report of analyses of the 1987 examination (5). All three models were implemented with and without covariate adjustment. Chapter 4 provides a more detailed discussion of the models.

RESULTS

Exposure Analysis

Physical Examination Variable

Skin Reaction Test

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

Under both the minimal and maximal assumptions, the unadjusted analysis of the composite skin reaction test displayed a nonsignificant negative association with initial dioxin (Table 16-3 [a] and [b]: $p=0.519$ and $p=0.207$, respectively).

Similarly, the adjusted analysis of the composite skin reaction test was not significant for an association with initial dioxin under either the minimal or the maximal assumption (Table 16-3 [c] and [d]: $p=0.201$ and $p=0.207$, respectively).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

Under both the minimal and maximal assumptions, the interaction between current dioxin and time since tour was not significant (Table 16-3 [e] and [f]: $p=0.474$ and $p=0.418$, respectively); hence, the relative risks were not significantly different between the two time strata. The relative risks for each time stratum were not significant.

Under the minimal assumption, the adjusted analysis contained a significant interaction among current dioxin, time, and age (Table 16-3 [g]: $p=0.013$). To investigate the interaction, adjusted analyses were performed separately for Ranch Hands born in or after 1942 and those born before 1942. For the younger Ranch Hands, the interaction of current dioxin and time was not significant (Appendix Table O-1: $p=0.198$). For the older Ranch Hands, the current dioxin-by-time interaction was significant ($p=0.024$). For older Ranch

TABLE 16-3.
Analysis of Composite Skin Test Diagnosis

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Percent Possibly Abnormal	Est. Relative Risk (95% C.I.) ^a	p-Value
a) Minimal (n=397)	Low	100	7.0	0.89 (0.63,1.27)	0.519
	Medium	203	7.4		
	High	94	4.3		
b) Maximal (n=570)	Low	139	9.4	0.85 (0.66,1.10)	0.207
	Medium	293	7.2		
	High	138	5.1		
Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted					
Assumption	Adj. Relative Risk (95% C.I.) ^a		p-Value	Covariate Remarks	
c) Minimal (n=394)	0.78 (0.53,1.15)		0.201	CSMOK*ALC (p=0.008)	
d) Maximal (n=570)	0.85 (0.66,1.10)		0.207	- -	

^aRelative risk for a twofold increase in dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-3. (Continued)
Analysis of Composite Skin Test Diagnosis

Ranch Hands - Log₂ (Current Dioxin) and Time - Unadjusted						
Assumption	Time (Yrs.)	Percent Possibly Abnormal/(n) Current Dioxin			Est. Relative Risk (95% C.I.) ^a	p-Value
		Low	Medium	High		
e) Minimal (n=397)	≤18.6	7.0 (57)	9.0 (100)	2.5 (40)	0.78 (0.44,1.39)	0.474 ^b 0.393 ^c
	>18.6	4.9 (41)	6.7 (104)	5.5 (55)	1.02 (0.64,1.61)	0.946 ^c
f) Maximal (n=570)	≤18.6	10.0 (80)	7.8 (142)	6.1 (66)	0.77 (0.52,1.15)	0.418 ^b 0.202 ^c
	>18.6	7.3 (69)	6.5 (138)	5.3 (75)	0.96 (0.68,1.35)	0.814 ^c
Ranch Hands - Log₂ (Current Dioxin) and Time - Adjusted						
Assumption	Time (Yrs.)	Adj. Relative Risk (95% C.I.) ^a		p-Value	Covariate Remarks	
g) Minimal (n=394)	≤18.6	0.64 (0.35,1.16)**		0.341** ^b 0.143** ^c	CURR*TIME*AGE (p=0.013) ALC*CSMOK (p=0.004)	
	>18.6	0.92 (0.55,1.54)**		0.762** ^c		
h) Maximal (n=570)	≤18.6	0.77 (0.52,1.15)		0.418 ^b 0.202 ^c	- -	
	>18.6	0.96 (0.68,1.35)		0.814 ^c		

^aRelative risk for a twofold increase in dioxin.

^bTest of significance for homogeneity of relative risks (current dioxin continuous, time categorized).

^cTest of significance for relative risk equal to 1 (current dioxin continuous, time categorized).

**Log₂ (current dioxin)-by-time-by-covariate interaction (0.01<p≤0.05); adjusted relative risk, confidence interval, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

CURR: Log₂ (current dioxin).

TIME: Time since tour.

TABLE 16-3. (Continued)
Analysis of Composite Skin Test Diagnosis

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Percent Possibly Abnormal	Contrast	Est. Relative Risk (95% C.I.)	p-Value
Background	605	4.6	All Categories		0.331
Unknown	269	7.1	Unknown vs. Background	1.57 (0.86,2.86)	0.143
Low	153	7.8	Low vs. Background	1.75 (0.87,3.53)	0.116
High	141	5.7	High vs. Background	1.24 (0.55,2.78)	0.603
Total	1,168				

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Contrast	Adj. Relative Risk (95% C.I.)	p-Value	Covariate Remarks
Background	604	All Categories		0.332	AGE (p=0.008) DRKYR (p=0.054)
Unknown	266	Unknown vs. Background	1.52 (0.83,2.78)	0.176	
Low	151	Low vs. Background	1.81 (0.89,3.67)	0.099	
High	137	High vs. Background	1.33 (0.56,3.15)	0.519	
Total	1,158				

Note: Background (Comparisons): Current Dioxin ≤ 10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤ 10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤ 33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

Hands with time since tour less than or equal to 18.6 years, there was a nonsignificant negative association with current dioxin and for those whose time was greater than 18.6 years, there was a nonsignificant positive association. Without the interaction of current dioxin, time, and age in the model, the adjusted relative risks were not significantly different between the two time strata (Table 16-3 [g]: $p=0.341$) and the adjusted risks within time strata also were not significant.

Under the maximal assumption, none of the covariates or associated interaction terms were retained in the adjusted analysis; therefore, the unadjusted and adjusted results are the same (as seen in Table 16-3 [f] and [h], respectively).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

In the unadjusted analysis of the relative frequency of participants with a possibly abnormal composite skin test reaction, the overall contrast of Ranch Hands classified in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category was nonsignificant (Table 16-3 [i]: $p=0.331$).

In the adjusted analysis of the composite skin test reaction, the overall contrast for Ranch Hands in the unknown, low, and high current dioxin categories versus Comparisons in the background current dioxin category was also nonsignificant (Table 16-3 [j]: $p=0.332$). The contrast for Ranch Hands in the low current dioxin category versus the Comparisons in the background current dioxin category was marginally significant ($p=0.099$, Adj. RR=1.81, 95% C.I.: [0.89,3.67]).

In the 1987 examination report, the composite skin test displayed unadjusted and adjusted relative risks that were greater than 1 for the Ranch Hand versus Comparison analyses. Although the relative risks of the three Ranch Hand versus Comparison contrasts were nonsignificant, each relative risk exceeded 1. The risks, however, were not indicative of a dose-response pattern.

Laboratory Examination Data: Quantitative Studies—Cell Surface Marker (Phenotypic) Studies

CD2 Cells

Model 1: Ranch Hands - Log₂(Initial Dioxin)

For the unadjusted analyses under both the minimal and maximal assumptions, the associations between CD2 cell counts and initial dioxin were not significant in the adjusted analysis (Table 16-4 [a] and [b]: $p=0.747$ and $p=0.628$, respectively).

Under the minimal assumption, the adjusted analysis contained a significant interaction between initial dioxin and current alcohol use (Table 16-4 [c]: $p=0.003$). Stratifying by current alcohol use (zero to one drink per day, over one drink per day), there was a significant negative association between CD2 cell counts and initial dioxin for Ranch Hands who had more than one drink per day (Appendix Table O-1: $p=0.002$). For the other current drinking stratum, there was a nonsignificant positive association ($p=0.442$). Under the maximal

TABLE 16-4.
Analysis of CD2 Cells (cells/mm³)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=195) (R ² <0.001)	Low	45	1,699.1	-0.007 (0.021)	0.747
	Medium	97	1,662.3		
	High	53	1,614.6		
b) Maximal (n=273) (R ² <0.001)	Low	65	1,645.5	0.008 (0.016)	0.628
	Medium	136	1,628.2		
	High	72	1,647.1		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=194) (R ² =0.125)	Low	45	****	****	****	INIT*ALC (p=0.003) AGE (p=0.091) CSMOK (p=0.008) DRKYR (p=0.036)
	Medium	96	****			
	High	53	****			
d) Maximal (n=272) (R ² =0.110)	Low	65	1,665.5	0.002 (0.016)	0.874	CSMOK (p<0.001) AGE*DRKYR (p<0.001)
	Medium	135	1,626.4			
	High	72	1,621.6			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD2 cells versus log₂ dioxin.

****Log₂ (initial dioxin)-by-covariate interaction (p≤0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

INIT: Log₂ (initial dioxin).

TABLE 16-4. (Continued)
Analysis of CD2 Cells (cells/mm³)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=195) (R ² =0.007)	≤18.6	1,640.5 (22)	1,602.2 (49)	1,626.4 (22)	-0.019 (0.033)	0.739 ^c 0.563 ^d	
	>18.6	1,737.4 (25)	1,697.2 (47)	1,659.0 (30)	-0.005 (0.028)	0.870 ^d	
f) Maximal (n=273) (R ² =0.004)	≤18.6	1,581.1 (39)	1,637.0 (70)	1,556.5 (30)	0.001 (0.024)	0.880 ^c 0.961 ^d	
	>18.6	1,630.8 (24)	1,659.9 (67)	1,718.0 (43)	0.006 (0.023)	0.786 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=194) (R ² =0.090)	≤18.6	1,679.4 (22)	1,598.4 (49)	1,588.9 (22)	-0.036 (0.033)	0.448 ^c 0.289 ^d	AGE (p=0.103) CSMOK (p=0.011) DRKYR (p=0.058)
	>18.6	1,742.8 (25)	1,665.6 (46)	1,684.1 (30)	-0.003 (0.029)	0.907 ^d	
h) Maximal (n=272) (R ² =0.126)	≤18.6	1,718.6 (39)	1,757.6 (70)	1,654.1 (30)	-0.007 (0.024)	0.717 ^c 0.773 ^d	CSMOK (p<0.001) AGE*DRKYR (p<0.001)
	>18.6	1,828.9 (24)	1,766.0 (66)	1,822.6 (43)	0.005 (0.022)	0.835 ^d	RACE*ALC (p=0.050)

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD2 cells versus log₂ dioxin.

^cTest of significance for current dioxin-by-time interaction (current dioxin continuous and time categorized).

^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-4. (Continued)
Analysis of CD2 Cells (cells/mm³)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted					
Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	307	1,615.9	All Categories		0.712
Unknown	130	1,568.6	Unknown vs. Background	-47.3 --	0.405
Low	74	1,636.2	Low vs. Background	20.3 --	0.777
High	76	1,651.5	High vs. Background	35.6 --	0.618
Total	587		(R ² =0.002)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted						
Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	306	1,687.0**	All Categories		0.825**	DXCAT*AGE (p=0.015)
Unknown	130	1,645.5**	Unknown vs. Background	-41.5 --**	0.470**	DXCAT*DRKYR (p=0.014)
Low	73	1,709.3**	Low vs. Background	22.3 --**	0.759**	RACE (p=0.112)
High	76	1,704.9**	High vs. Background	17.9 --**	0.805**	CSMOK (p<0.001)
Total	585		(R ² =0.106)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fP-value is based on difference of means on natural logarithm scale.

**Categorized current dioxin-by-covariate interaction (0.01<p≤0.05); adjusted mean and p-value derived from a model fitted after deletion of this interaction.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
 Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
 Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
 High (Ranch Hands): Current Dioxin >33.3 ppt.
 DXCAT: Categorized current dioxin.

assumption, the association between CD2 cells and initial dioxin was not significant (Table 16-4 [d]: $p=0.874$).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis relating CD2 cells to current dioxin and time since tour, the models under both the minimal and maximal assumptions did not contain significant current dioxin-by-time interactions (Table 16-4 [e] and [f]: $p=0.739$ and $p=0.880$, respectively), indicating that the relationships between CD2 and current dioxin did not differ between time strata. In the adjusted analysis, the models based on the minimal and maximal assumptions also contained nonsignificant current dioxin-by-time interactions (Table 16-4 [g] and [h]: $p=0.448$ and $p=0.717$, respectively).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

In the unadjusted analysis of CD2 cell counts, the mean levels of the four current dioxin categories did not differ significantly (Table 16-4 [i]: $p=0.712$).

The adjusted analysis of the CD2 cell counts displayed a significant interaction between categorized current dioxin and age and a significant interaction between categorized current dioxin and lifetime alcohol history (Table 16-4 [j]: $p=0.015$ and $p=0.014$, respectively). To investigate the interactions, age was dichotomized for Ranch Hands and Comparisons born in or after 1942 and those born prior to 1942 and lifetime alcohol history was trichotomized as 0 drink-years, greater than 0 but less than 40 drink-years, and over 40 drink-years. For participants born in or after 1942 with a lifetime alcohol history of zero drink-years, the high versus background contrast was of borderline significance (Appendix Table O-1: $p=0.082$) with the Comparisons having a higher adjusted CD2 mean than the Ranch Hands. However, the contrast was based on eight Comparisons and three Ranch Hands. For participants born in or after 1942 with a lifetime alcohol history of greater than 0 but less than 40 drink-years, the unknown versus background contrast was significant ($p=0.032$), with the Comparisons having the higher adjusted CD2 mean. All other contrasts were nonsignificant. A followup model was examined without the two interactions cited above. For that model, the overall contrast was nonsignificant (Table 16-4 [j]: $p=0.825$).

CD4 Cells

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

Under both the minimal and the maximal assumptions, the unadjusted analyses of the CD4 cell counts were not significant for an association with initial dioxin (Table 16-5 [a] and [b]: $p=0.809$ and $p=0.157$, respectively). For the adjusted analyses, the minimal and maximal assumptions also exhibited nonsignificant associations between the CD4 cell counts and initial dioxin (Table 16-5 [c] and [d]: $p=0.936$ and $p=0.324$, respectively).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

For the unadjusted analyses of CD4, under both the minimal and maximal assumptions, the interaction of current dioxin and time since tour was not significant (Table 16-5 [e] and [f]: $p=0.510$ and $p=0.453$, respectively). Therefore, the associations (i.e., slopes) did not differ significantly between the two time strata.

TABLE 16-5.
Analysis of CD4 Cells (cells/mm³)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=194) (R ² <0.001)	Low	45	940.4	0.006 (0.024)	0.809
	Medium	96	966.3		
	High	53	920.2		
b) Maximal (n=272) (R ² =0.007)	Low	65	894.5	0.027 (0.019)	0.157
	Medium	136	924.5		
	High	71	941.0		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	Adj. n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=193) (R ² =0.126)	Low	45	960.6	0.002 (0.024)	0.936	AGE (p=0.097) CSMOK (p<0.001) DRKYR (p=0.063)
	Medium	95	952.7			
	High	53	920.6			
d) Maximal (n=271) (R ² =0.155)	Low	65	921.1	0.018 (0.019)	0.324	AGE*CSMOK (p=0.005) AGE*PACKYR (p=0.042) AGE*DRKYR (p<0.001) CSMOK*PACKYR (p=0.039)
	Medium	135	917.7			
	High	71	923.6			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD4 cells versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-5. (Continued)
Analysis of CD4 Cells (cells/mm³)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=194) (R ² =0.008)	≤18.6	943.6 (22)	917.8 (48)	902.2 (22)	-0.017 (0.038)	0.510 ^c 0.657 ^d	
	>18.6	941.2 (25)	999.4 (47)	959.0 (30)	0.016 (0.032)	0.621 ^d	
f) Maximal (n=272) (R ² =0.009)	≤18.6	880.9 (39)	939.2 (70)	865.2 (29)	0.008 (0.029)	0.453 ^c 0.772 ^d	
	>18.6	840.3 (24)	935.9 (67)	997.9 (43)	0.038 (0.027)	0.161 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=193) (R ² =0.170)	≤18.6	886.9** (22)	822.8** (48)	781.9** (22)	-0.042 (0.037)**	0.213*** ^c 0.259*** ^d	CURR*TIME*DRKYR (p=0.038) AGE (p=0.053)
	>18.6	864.0** (25)	887.2** (46)	894.1** (30)	0.017 (0.031)**	0.598*** ^d	RACE (p=0.135) CSMOK (p<0.001)
h) Maximal (n=271) (R ² =0.182)	≤18.6	922.0** (39)	929.8** (70)	845.6** (29)	-0.008 (0.028)**	0.243*** ^c 0.778*** ^d	CURR*TIME*AGE (p=0.024) AGE*CSMOK (p=0.003)
	>18.6	876.3** (24)	925.9** (66)	972.3** (43)	0.036 (0.026)**	0.174*** ^d	AGE*PACKYR (p=0.038) AGE*DRKYR (p<0.001) CSMOK*PACKYR (p=0.030)

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD4 cells versus log₂ dioxin.

^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).

**Log₂ (current dioxin)-by-time-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-5. (Continued)
Analysis of CD4 Cells (cells/mm³)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	301	907.8	All Categories		0.351
Unknown	127	861.5	Unknown vs. Background	-46.3 --	0.216
Low	72	938.6	Low vs. Background	30.8 --	0.525
High	72	942.2	High vs. Background	34.4 --	0.478
Total	572		(R ² =0.006)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	301	907.1	All Categories		0.406	AGE (p=0.018) CSMOK (p<0.001)
Unknown	127	866.5	Unknown vs. Background	-40.6 --	0.259	
Low	72	945.4	Low vs. Background	38.3 --	0.409	
High	72	929.0	High vs. Background	21.9 --	0.637	
Total	572		(R ² =0.095)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fP-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

For the adjusted analyses of CD4 cell counts under the minimal assumption, there was a significant interaction among current dioxin, time, and lifetime alcohol history (Table 16-5 [g]: $p=0.038$). To examine the interaction, Ranch Hands with lifetime alcohol history values were dichotomized into less than or equal to 40 drink-years or greater than 40 drink-years. For the former lifetime alcohol history stratum, the interaction between current dioxin and time was significant (Appendix Table O-1: $p=0.013$); there was a significant negative association ($p=0.035$) with current dioxin for time less than or equal to 18.6 years, and a nonsignificant positive association with current dioxin for time over 18.6 years ($p=0.200$). For the latter lifetime alcohol history stratum, the interaction of current dioxin and time was marginally significant (Appendix Table O-1: $p=0.054$) with a nonsignificant positive association between CD4 cells and current dioxin for time of 18.6 years or less and a nonsignificant negative association for time over 18.6 years ($p=0.191$ and $p=0.163$, respectively). Without the interaction of current dioxin, time, and lifetime alcohol history in the adjusted model, the interaction between current dioxin and time was not significant (Table 16-5 [g]: $p=0.213$).

Under the maximal assumption, the adjusted analysis contained a significant interaction among current dioxin, time, and age (Table 16-5 [h]: $p=0.024$). The interaction was explored for Ranch Hands born in or after 1942 and those born prior to 1942. For the older Ranch Hands, the association between CD4 and current dioxin differed significantly between the time strata (Appendix Table O-1: $p=0.043$); for time of 18.6 years or less there was a nonsignificant negative association ($p=0.114$), and for time greater than 18.6 years there was a nonsignificant positive association ($p=0.207$). For the younger Ranch Hands, the interaction of current dioxin and time was nonsignificant for CD4 ($p=0.753$). An adjusted model without the interaction of current dioxin, time, and age displayed a nonsignificant current dioxin-by-time interaction (Table 16-5 [h]: $p=0.243$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the unadjusted and the adjusted analysis of CD4 cell counts, the overall contrasts of the four current dioxin categories were not significant (Table 16-5 [i] and [j]: $p=0.351$ and $p=0.406$, respectively) and none of the Ranch Hand versus Comparison contrasts were significant ($p>0.20$ for all).

CD8 Cells

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

For both the minimal and the maximal assumptions, the association between the CD8 cell counts and initial dioxin was not significant in the unadjusted analysis (Table 16-6 [a] and [b]: $p=0.934$ and $p=0.705$, respectively).

Under the minimal assumption, the adjusted analysis of the CD8 cell counts contained a significant interaction between initial dioxin and lifetime alcohol history (Table 16-6 [c]: $p<0.001$). The interaction was investigated by trichotomizing the Ranch Hands into the following lifetime alcohol history strata: 0 drink-years, above 0 drink-years to 40 drink-years, and over 40 drink-years. For Ranch Hands with a lifetime history over 40 drink-years, there was a significant negative association between CD8 cell counts and initial dioxin (Appendix Table O-1: $p=0.016$). For the nondrinkers, there was a nonsignificant positive

TABLE 16-6.
Analysis of CD8 Cells (cells/mm³)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=194) (R ² <0.001)	Low	45	494.6	-0.002 (0.029)	0.934
	Medium	96	468.9		
	High	53	483.4		
b) Maximal (n=271) (R ² =0.001)	Low	64	505.7	-0.008 (0.021)	0.705
	Medium	135	475.2		
	High	72	486.7		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=193) (R ² =0.142)	Low	45	****	****	****	INIT*DRKYR (p<0.001) PACKYR (p=0.086) AGE*RACE (p=0.047) AGE*DRKYR (p=0.001) CSMOK*DRKYR (p=0.014)
	Medium	95	****			
	High	53	****			
d) Maximal (n=270) (R ² =0.076)	Low	64	511.9**	-0.014 (0.022)**	0.518**	INIT*ALC (p=0.041) CSMOK (p=0.011) AGE*DRKYR (p=0.003)
	Medium	134	473.9**			
	High	72	479.5**			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD8 cells versus log₂ dioxin.

**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deleting this interaction.

****Log₂ (initial dioxin)-by-covariate interaction (p≤0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-6. (Continued)
Analysis of CD8 Cells (cells/mm³)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=194) (R ² =0.005)	≤18.6	456.7 (22)	444.6 (49)	518.6 (22)	-0.009 (0.045)	0.982 ^c 0.840 ^d	
	>18.6	529.7 (24)	479.2 (47)	485.4 (30)	-0.010 (0.039)	0.788 ^d	
f) Maximal (n=271) (R ² =0.013)	≤18.6	466.7 (39)	465.2 (70)	464.9 (30)	-0.003 (0.032)	0.472 ^c 0.931 ^d	
	>18.6	590.8 (23)	489.1 (66)	493.6 (43)	-0.035 (0.030)	0.257 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=193) (R ² =0.059)	≤18.6	472.1 (22)	443.1 (49)	511.3 (22)	-0.029 (0.046)	0.721 ^c 0.531 ^d	AGE (p=0.047) PACKYR (p=0.045) DRKYR (p=0.107)
	>18.6	523.5 (24)	470.2 (46)	489.6 (30)	-0.008 (0.040)	0.842 ^d	
h) Maximal (n=270) (R ² =0.073)	≤18.6	476.9 (39)	462.8 (70)	461.2 (30)	-0.013 (0.032)	0.513 ^c 0.686 ^d	CSMOK (p=0.013) AGE*DRKYR (p=0.004)
	>18.6	620.1 (23)	483.0 (65)	480.4 (43)	-0.041 (0.030)	0.174 ^d	

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD8 cells versus log₂ dioxin.

^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-6. (Continued)
Analysis of CD8 Cells (cells/mm³)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	301	471.8	All Categories		0.937
Unknown	126	485.2	Unknown vs. Background	13.4 --	0.581
Low	72	469.2	Low vs. Background	-2.6 --	0.930
High	73	481.6	High vs. Background	9.8 --	0.741
Total	572		(R ² <0.001)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	301	473.1	All Categories		0.937	AGE (p=0.089) CSMOK (p<0.001)
Unknown	126	485.2	Unknown vs. Background	12.1 --	0.614	ALC (p=0.144)
Low	71	465.3	Low vs. Background	-7.8 --	0.790	
High	73	475.5	High vs. Background	2.4 --	0.934	
Total	571		(R ² =0.037)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fp-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

association ($p=0.760$) and for the 0 drink-years to 40 drink-years stratum there was a nonsignificant negative association ($p=0.894$).

Under the maximal assumption, the adjusted analysis of the CD8 cell counts contained a significant interaction between initial dioxin and current alcohol use (Table 16-6 [d]: $p=0.041$). After stratifying the Ranch Hands into two current alcohol use strata (zero to one drink per day, over one drink per day), a significant negative association was found between CD8 cell counts and initial dioxin for Ranch Hands who had more than one drink per day (Appendix Table O-1: $p=0.033$). The other stratum exhibited a nonsignificant positive association ($p=0.844$). Deleting the initial dioxin-by-current alcohol use interaction from the model resulted in a nonsignificant association (Table 16-6 [d]: $p=0.518$) between CD8 and initial dioxin.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

For both the minimal and maximal assumptions, the unadjusted analysis of CD8 cell counts indicated the associations with current dioxin did not differ significantly between time since tour strata (Table 16-6 [e] and [f]: $p=0.982$ and $p=0.472$, respectively).

Under the minimal and maximal assumptions, the adjusted slopes for the association between CD8 cells and current dioxin did not differ significantly between time strata (Table 16-6 [g] and [h]: $p=0.721$ and $p=0.513$, respectively).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the unadjusted and adjusted analyses of CD8 cell counts, the overall contrast of the four current dioxin categories was not significant (Table 16-6 [i] and [j]: $p=0.937$ for each).

CD20 Cells

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

For the unadjusted analyses under both the minimal and maximal assumptions, the association between CD20 cell counts and initial dioxin was not significant (Table 16-7 [a] and [b]: $p=0.102$ and $p=0.212$, respectively).

In the adjusted analysis under the minimal assumption, there was a significant interaction between initial dioxin and age (Table 16-7 [c]: $p=0.013$). Current cigarette smoking, current alcohol use, and lifetime alcohol history were covariates retained in the adjusted model. To investigate the interaction, the results were examined separately for Ranch Hands born in or after 1942 and those Ranch Hands born prior to 1942. For the older Ranch Hands, there was a significant positive association between CD20 cell counts and initial dioxin (Appendix Table O-1: $p=0.002$). For the younger Ranch Hands, there was a nonsignificant negative association ($p=0.566$). Without the interaction of initial dioxin and age in the model, there was a positive association between the CD20 cell counts and initial dioxin that was marginally significant (Table 16-7 [c]: $p=0.086$).

In the adjusted analysis of the maximal cohort, the association between CD20 and initial dioxin was not significant (Table 16-7 [d]: $p=0.363$).

TABLE 16-7.
Analysis of CD20 Cells (cells/mm³)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=195) (R ² =0.389)	Low	45	127.7	0.074 (0.045)	0.102
	Medium	97	166.6		
	High	53	169.6		
b) Maximal (n=273) (R ² =0.341)	Low	65	157.9	0.036 (0.029)	0.212
	Medium	136	144.7		
	High	72	164.6		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=194) (R ² =0.516)	Low	45	142.8**	0.075 (0.043)**	0.086**	INIT*AGE (p=0.013) CSMOK (p=0.064) ALC (p=0.055) DRKYR (p=0.004)
	Medium	96	155.0**			
	High	53	167.3**			
d) Maximal (n=272) (R ² =0.465)	Low	65	196.1	0.025 (0.027)	0.363	RACE (p=0.033) CSMOK (p=0.001) ALC (p=0.072) AGE*DRKYR (p=0.002)
	Medium	135	174.3			
	High	72	193.7			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD20 cells versus log₂ dioxin.

**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-7. (Continued)
Analysis of CD20 Cells (cells/mm³)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=195) (R ² =0.388)	≤18.6	122.8 (22)	161.0 (49)	168.3 (22)	0.052 (0.066)	0.825 ^c 0.436 ^d	
	>18.6	140.4 (25)	168.7 (47)	175.5 (30)	0.071 (0.063)	0.262 ^d	
f) Maximal (n=273) (R ² =0.345)	≤18.6	162.9 (39)	151.6 (70)	152.3 (30)	0.007 (0.043)	0.302 ^c 0.877 ^d	
	>18.6	140.3 (24)	142.5 (67)	170.6 (43)	0.069 (0.042)	0.099 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=194) (R ² =0.485)	≤18.6	128.2 (22)	165.2 (49)	173.9 (22)	0.047 (0.061)	0.371 ^c 0.446 ^d	CSMOK (p=0.023) ALC (p=0.041) DRKYR (p=0.004)
	>18.6	132.7 (25)	155.9 (46)	192.2 (30)	0.121 (0.059)	0.043 ^d	
h) Maximal (n=272) (R ² =0.473)	≤18.6	203.4 (39)	186.5 (70)	186.7 (30)	-0.008 (0.040)	0.171 ^c 0.845 ^d	RACE (p=0.032) CSMOK (p=0.001) ALC (p=0.051)
	>18.6	187.5 (24)	164.4 (66)	202.2 (43)	0.068 (0.039)	0.083 ^d	AGE*DRKYR (p=0.003)

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD20 cells versus log₂ dioxin.

^cTest of significance for current dioxin-by-time interaction (current dioxin continuous and time categorized).

^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-7. (Continued)
Analysis of CD20 Cells (cells/mm³)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	301	148.9	All Categories		0.269
Unknown	127	154.3	Unknown vs. Background	5.4 --	0.544
Low	72	161.7	Low vs. Background	12.8 --	0.260
High	73	171.1	High vs. Background	22.2 --	0.066
Total	573		(R ² =0.213)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	301	172.4**	All Categories		0.485**	DXCAT*AGE (p=0.014) RACE (p=0.004)
Unknown	127	176.5**	Unknown vs. Background	4.1 --**	0.670**	CSMOK (p<0.001)
Low	71	183.2**	Low vs. Background	10.8 --**	0.377**	ALC (p=0.008)
High	73	190.8**	High vs. Background	18.4 --**	0.148**	
Total	572		(R ² =0.344)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fp-value is based on difference of means on natural logarithm scale.

**Categorized current dioxin-by-covariate interaction (0.01<p≤0.05); adjusted mean, and p-value derived from a model fitted after deletion of this interaction.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of the relationship between CD20 cell counts with current dioxin and time since tour, the interaction of current dioxin and time was not significant for both assumptions (Table 16-7 [e] and [f]: $p=0.825$ and $p=0.302$, respectively). Under the maximal assumption, Ranch Hands with early tours (i.e., time > 18.6 years), displayed a marginally significant positive association between CD20 and current dioxin ($p=0.099$).

Under the minimal assumption, the adjusted analysis of the association between CD20 cells with current dioxin and time indicated that the interaction between current dioxin and time was not significant (Table 16-7 [g]: $p=0.371$). Current cigarette smoking, current alcohol use, and lifetime alcohol history were covariates retained in the adjusted model. For Ranch Hands with time over 18.6 years, there was a significant positive association between CD20 cells and current dioxin ($p=0.043$).

Under the maximal assumption, the adjusted analysis of the CD20 cell counts also indicated that the interaction between current dioxin and time was not significant (Table 16-7 [h]: $p=0.171$). Therefore, the adjusted slopes for the association between CD20 cells and current dioxin were not significantly different between time strata. For Ranch Hands with time over 18.6 years, there was a positive relationship between CD20 cells and current dioxin that was marginally significant (Table 16-7 [h]: $p=0.083$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The simultaneous contrast of the CD20 unadjusted means for the four current dioxin categories was nonsignificant (Table 16-7 [i]: $p=0.269$). The unadjusted means for the background, unknown, low, and high current dioxin categories were 148.9, 154.3, 161.7, and 171.1 cell/mm³. The CD20 mean for Ranch Hands with high current dioxin was marginally higher than the CD20 mean of the Comparisons ($p=0.066$).

In the adjusted analysis of the CD20 cell counts using the four categories, there was a significant interaction between categorized current dioxin and age (Table 16-7 [j]: $p=0.014$). To explore the interaction, the results were examined separately for Ranch Hands and Comparisons born in or after 1942 and those born prior to 1942 (Appendix Table O-1). For the younger participants, the overall contrast of the adjusted CD20 cell means was not significant ($p=0.307$); however, all Ranch Hand categories had lower adjusted mean CD20 counts than Comparisons and Ranch Hands with unknown current dioxin had a marginally lower adjusted mean count ($p=0.069$; 170.2 versus 200.4 cells/mm³). For the older participants, the overall contrast of the adjusted CD20 cell means for the four current dioxin categories was significant ($p=0.006$). The adjusted means for the background, unknown, low, and high current dioxin were 156.9, 179.9, 186.3, and 215.9 cells/mm³. The three contrasts were at least marginally significant (unknown versus background, $p=0.047$; low versus background, $p=0.053$; high versus background, $p=0.002$). A followup model without the interaction of age and categorized current dioxin displayed a nonsignificant overall contrast (Table 16-7 [j]: $p=0.485$) and individual contrasts ($p>0.10$ for all).

CD14 Cells

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

The unadjusted analysis of the CD14 cell counts exhibited nonsignificant associations with initial dioxin for both the minimal and the maximal assumptions (Table 16-8 [a] and [b]: $p=0.842$ and $p=0.633$, respectively).

In the adjusted analysis of the CD14 cells under the minimal assumption, the model contained significant interactions between initial dioxin and lifetime smoking history, and between initial dioxin and current alcohol use (Table 16-8 [c]: $p=0.014$ and $p=0.008$, respectively). To investigate these interactions, lifetime smoking history was dichotomized into zero pack-years and over zero pack-years, and current alcohol use was dichotomized into zero to one drink per day, and over one drink per day. For Ranch Hands who smoked and had one drink per day or less, there was a marginally significant positive association between CD14 cells and initial dioxin (Appendix Table O-1: $p=0.051$). For Ranch Hands who smoked and had more than one drink per day, there was a marginally significant negative association ($p=0.078$). For the other strata combinations of lifetime smoking and current alcohol use, there were nonsignificant negative associations between CD14 and initial dioxin ($p>0.25$ for both).

In the adjusted analysis under the maximal assumption, the association between CD14 and initial dioxin was nonsignificant (Table 16-8 [d]: $p=0.728$).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of the relationship between CD14 cell counts with current dioxin and time since tour, the interaction of current dioxin and time was not significant for both the minimal and maximal assumptions (Table 16-8 [e] and [f]: $p=0.156$ and $p=0.300$); thus, the association between CD14 cells and current dioxin did not differ significantly between time strata.

In the adjusted analysis of the CD14 cell counts under the minimal assumption, the interaction between current dioxin and time was nonsignificant (Table 16-8 [g]: $p=0.174$).

In the adjusted analysis of the CD14 cell counts under the maximal assumption, there was a significant interaction among current dioxin, time, and lifetime cigarette smoking history (Table 16-8 [h]: $p=0.001$). Because of the interaction, the association between CD14 cell counts and current dioxin within each time strata was investigated for Ranch Hands categorized by lifetime cigarette smoking history (0 pack-years, over 0 pack-years but not over 10 pack-years, and over 10 pack-years). For nonsmoker Ranch Hands and Ranch Hands not exceeding 10 pack-years, the current dioxin-by-time interaction was nonsignificant (Appendix Table O-1: $p=0.309$ and $p=0.841$, respectively). For Ranch Hands with more than 10 pack-years for lifetime cigarette smoking history, the association between CD14 and current dioxin differed significantly between time strata ($p=0.014$). Within that lifetime smoking stratum, there was a significant positive association between CD14 and current dioxin for Ranch Hands with time over 18.6 years ($p=0.006$) and a nonsignificant negative association for the other time strata ($p=0.452$).

TABLE 16-8.
Analysis of CD14 Cells (cells/mm³)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=197) (R ² =0.651)	Low	45	29.7	0.011 (0.054)	0.842
	Medium	98	31.1		
	High	54	29.6		
b) Maximal (n=275) (R ² =0.568)	Low	65	31.2	-0.017 (0.036)	0.633
	Medium	137	28.9		
	High	73	27.6		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=196) (R ² =0.728)	Low	45	****	****	****	INIT*PACKYR (p=0.014) INIT*ALC (p=0.008) RACE (p=0.032) CSMOK*PACKYR (p=0.015) CSMOK*ALC (p=0.009)
	Medium	97	****			
	High	54	****			
d) Maximal (n=274) (R ² =0.596)	Low	65	32.4	-0.012 (0.036)	0.728	DRKYR (p=0.147) CSMOK (p=0.013)
	Medium	136	28.4			
	High	73	28.2			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD14 cells versus log₂ dioxin.

****Log₂ (initial dioxin)-by-covariate interaction (p≤0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-8. (Continued)
Analysis of CD14 Cells (cells/mm³)

Ranch Hands - Log₂ (Current Dioxin) and Time - Unadjusted

Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value
		Low	Medium	High		
e) Minimal (n=197) (R ² =0.662)	≤18.6	30.0 (22)	35.1 (49)	28.1 (23)	-0.045 (0.077)	0.156 ^c 0.559 ^d
	>18.6	23.4 (25)	30.3 (48)	32.7 (30)	0.111 (0.079)	0.161 ^d
f) Maximal (n=275) (R ² =0.573)	≤18.6	29.5 (39)	34.0 (70)	24.8 (31)	-0.046 (0.056)	0.300 ^c 0.415 ^d
	>18.6	26.4 (24)	26.8 (68)	31.3 (43)	0.039 (0.056)	0.488 ^d

Ranch Hands - Log₂ (Current Dioxin) and Time - Adjusted

Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=197) (R ² =0.682)	≤18.6	29.2 (22)	35.5 (49)	29.5 (23)	-0.019 (0.076)	0.174 ^c 0.801 ^d	CSMOK (p=0.018)
	>18.6	23.6 (25)	29.7 (48)	34.2 (30)	0.127 (0.077)	0.102 ^d	
h) Maximal (n=274) (R ² =0.629)	≤18.6	**** (39)	**** (70)	**** (31)	****	****	CURR*TIME*PACKYR (p=0.001) DRKYR (p=0.085)
	>18.6	**** (24)	**** (67)	**** (43)	****	****	

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD14 cells versus log₂ dioxin.

^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).

****Log₂ (current dioxin)-by-time-by-covariate interaction (p<0.01); adjusted mean, adjusted slope, confidence interval, and p-value not presented.

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-8. (Continued)
Analysis of CD14 Cells (cells/mm³)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	301	32.7	All Categories		0.674
Unknown	127	32.2	Unknown vs. Background	-0.5 --	0.844
Low	73	30.4	Low vs. Background	-2.3 --	0.476
High	74	29.1	High vs. Background	-3.6 --	0.260
Total	575		(R ² =0.348)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	301	29.3	All Categories		0.896	AGE*CSMOK (p=0.026) AGE*PACKYR (p=0.006)
Unknown	127	28.4	Unknown vs. Background	-0.9 --	0.705	CSMOK*PACKYR
Low	73	27.9	Low vs. Background	-1.4 --	0.612	(p<0.001)
High	74	27.4	High vs. Background	-1.9 --	0.504	RACE (p=0.080)
Total	575		(R ² =0.423)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fp-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the unadjusted and adjusted analysis of CD14 cell counts, the overall contrasts of the four current dioxin categories were not significant (Table 16-8 [i] and [j]: $p=0.674$ and $p=0.896$, respectively).

CD25 Cells

The CD25 cell counts consisted of both zero and nonzero cell counts. For the minimal and maximal cohorts approximately 30 percent of the CD25 values were zero. As a preliminary analysis to the unadjusted and adjusted analyses of the nonzero CD25 cell counts, the relative frequencies of CD25 values reported as zero were compared across the three initial dioxin categories (i.e., low, medium, and high initial dioxin). Under both assumptions, the relative frequencies were not significantly different among the initial dioxin categories (minimal, $p=0.279$; maximal, $p=0.220$). Relative frequencies of CD25 values reported as zero were also compared across the six combinations of three current dioxin categories (low, medium, and high) and the two time since tour strata (≤ 18.6 years, >18.6 years). For both cohorts, the relative frequencies of CD25 zero values were not significantly different across the six current dioxin and time combinations (minimal, $p=0.549$; maximal, $p=0.528$). Finally, the relative frequencies of CD25 values reported as zero were compared for Ranch Hands with unknown, low, and high current dioxin and Comparisons with background current dioxin. The frequencies were not significantly different ($p=0.781$).

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of the nonzero CD25 cell counts, the association with initial dioxin was not significant for both the minimal and maximal assumptions (Table 16-9 [a] and [b]: $p=0.339$ and $p=0.933$, respectively).

For the nonzero CD25 cell counts, the adjusted models for both the minimal and maximal assumptions contained interactions of initial dioxin with current cigarette smoking (Table 16-9 [c]: $p=0.004$, Table 16-9 [d]: $p=0.009$), lifetime cigarette smoking history (Table 16-9 [c]: $p=0.032$, Table 16-9 [d]: $p=0.001$), and lifetime alcohol history (Table 16-9 [c]: $p<0.001$, Table 16-9 [d]: $p=0.023$).

To explore these interactions, current cigarette smoking was dichotomized into nonsmokers (never smoked and former smokers combined) and smokers, lifetime cigarette smoking history was dichotomized as 10 pack-years or less and over 10 pack-years, and lifetime alcohol history was dichotomized as 40 drink-years or less and over 40 drink-years. Under the minimal assumption, each of the eight strata combinations of current cigarette smoking, lifetime cigarette smoking, and lifetime alcohol history displayed nonsignificant associations between CD25 and initial dioxin (Appendix Table O-1). Under the maximal assumption, there was a significant positive association between CD25 and initial dioxin for smokers with 10 pack-years or less of lifetime cigarette smoking and 40 drink-years or less on lifetime alcohol history ($p=0.003$). A marginally significant positive association was found for smokers with 10 pack-years or less of lifetime cigarette smoking and over 40 drink-years on lifetime alcohol history ($p=0.091$). For the other six strata combinations, the associations were nonsignificant.

TABLE 16-9.
Analysis of CD25 Cells (cells/mm³)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=140) (R ² =0.715)	Low	28	13.3	-0.096 (0.100)	0.339
	Medium	71	10.1		
	High	41	10.1		
b) Maximal (n=191) (R ² =0.665)	Low	43	12.1	0.006 (0.070)	0.933
	Medium	92	11.3		
	High	56	11.9		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=139) (R ² =0.819)	Low	28	****	****	****	INIT*CSMOK (p=0.004) INIT*PACKYR (p=0.032) INIT*DRKYR (p<0.001) RACE (p=0.056)
	Medium	70	****			
	High	41	****			
d) Maximal (n=190) (R ² =0.735)	Low	43	****	****	****	INIT*CSMOK (p=0.009) INIT*PACKYR (p=0.001) INIT*DRKYR (p=0.023) RACE (p=0.135)
	Medium	91	****			
	High	56	****			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD25 cells versus log₂ dioxin.

****Log₂ (initial dioxin)-by-covariate interaction (p≤0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-9. (Continued)
Analysis of CD25 Cells (cells/mm³)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=140) (R ² =0.738)	≤18.6	22.5 (12)	9.7 (36)	5.9 (17)	-0.351 (0.155)	0.051 ^c 0.028 ^d	
	>18.6	9.3 (17)	10.8 (35)	14.0 (23)	0.070 (0.141)	0.624 ^d	
f) Maximal (n=191) (R ² =0.670)	≤18.6	10.0 (27)	12.7 (44)	9.4 (24)	-0.091 (0.110)	0.314 ^c 0.413 ^d	
	>18.6	16.6 (15)	10.7 (48)	13.0 (33)	0.070 (0.110)	0.524 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=140) (R ² =0.738)	≤18.6	22.5 (12)	9.7 (36)	5.9 (17)	-0.351 (0.155)	0.051 ^c 0.028 ^d	--
	>18.6	9.3 (17)	10.8 (35)	14.0 (23)	0.070 (0.141)	0.624 ^d	
h) Maximal (n=191) (R ² =0.689)	≤18.6	10.4 (27)	12.0 (44)	9.5 (24)	-0.100 (0.109)	0.186 ^c 0.361 ^d	CSMOK*PACKYR (p=0.035)
	>18.6	13.7 (15)	10.7 (48)	13.5 (33)	0.114 (0.113)	0.317 ^d	

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD25 cells versus log₂ dioxin.

^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-9. (Continued)
Analysis of CD25 Cells (cells/mm³)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted						
Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e		p-Value ^f
Background	214	11.0	All Categories			0.612
Unknown	90	13.0	Unknown vs. Background	2.0 --		0.221
Low	51	10.4	Low vs. Background	-0.6 --		0.770
High	57	11.5	High vs. Background	0.5 --		0.760
Total	412		(R ² =0.538)			

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted						
Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e		p-Value ^f
Background	214	10.9	All Categories			0.603
Unknown	90	13.0	Unknown vs. Background	2.1 --		0.203
Low	51	10.6	Low vs. Background	-0.3 --		0.839
High	57	11.5	High vs. Background	0.6 --		0.761
Total	412		(R ² =0.546)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fP-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of the nonzero CD25 cells under the minimal assumption, the interaction of current dioxin and time since tour was marginally significant (Table 16-9 [e]: $p=0.051$); therefore the slopes were marginally significant between the two time strata. For time less than or equal to 18.6 years, there was a significant negative association between the CD25 cell counts and current dioxin ($p=0.028$). For this time stratum, the unadjusted CD25 means for low, medium, and high current dioxin were 22.5, 9.7, and 5.9 cells/mm³. For the other time stratum, there was a nonsignificant positive association ($p=0.624$).

Under the maximal assumption, the unadjusted analysis of nonzero CD25 cell counts had a nonsignificant interaction between current dioxin and time (Table 16-9 [f]: $p=0.314$); therefore, the slopes between the two time strata did not differ significantly.

In the adjusted analysis of nonzero CD25 cells under the minimal assumption, none of the covariates or interactions were retained in the model and therefore the unadjusted and adjusted results are the same for this cohort (as seen in Table 16-9 [e] and [g], respectively).

In the adjusted analysis of the nonzero CD25 cells under the maximal assumption, the interaction between current dioxin and time was not significant (Table 16-9 [h]: $p=0.186$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the unadjusted and adjusted analysis of the nonzero CD25 cell counts, the overall contrast of the four current dioxin categories was not significant (Table 16-9 [i] and [j]: $p=0.612$ and $p=0.603$, respectively).

HLA-DR Cells

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of HLA-DR cells, the association with initial dioxin was not significant under both the minimal and maximal assumptions (Table 16-10 [a] and [b]: $p=0.848$ and $p=0.960$).

Under the minimal assumption, the adjusted analysis contained a significant interaction between initial dioxin and age (Table 16-10 [c]: $p=0.002$). To investigate the interaction, adjusted analyses were performed for Ranch Hands born in or after 1942 and those born before 1942. For the younger Ranch Hands, there was a significant negative association between HLA-DR cells and initial dioxin (Appendix Table O-1: $p=0.020$). In contrast, there was a significant positive association, for the older Ranch Hands, between HLA-DR cells and initial dioxin ($p=0.050$).

Under the maximal assumption, the adjusted analysis contained significant interactions between initial dioxin and age, and initial dioxin and current alcohol use (Table 16-10 [d]: $p=0.025$ and $p=0.029$, respectively). To explore the interactions, age was dichotomized for participants born in or after 1942 and those born before 1942, and current alcohol use was dichotomized for participants having one drink or less per day and over one drink per day.

TABLE 16-10.

Analysis of HLA-DR Cells (cells/mm³)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=197) (R ² =0.573)	Low	45	417.4	-0.007 (0.034)	0.848
	Medium	98	461.4		
	High	54	410.4		
b) Maximal (n=275) (R ² =0.540)	Low	65	437.0	0.001 (0.023)	0.960
	Medium	137	427.6		
	High	73	422.3		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=196) (R ² =0.674)	Low	45	****	****	****	INIT*AGE (p=0.002)
	Medium	97	****			ALC (p=0.075)
	High	54	****			DRKYR (p=0.052)
d) Maximal (n=274) (R ² =0.644)	Low	65	451.9**	0.002 (0.022)**	0.943**	INIT*AGE (p=0.025)
	Medium	136	421.1**			INIT*ALC (p=0.029)
	High	73	427.4**			CSMOK (p=0.002) DRKYR (p=0.015)

^aTransformed from natural logarithm scale.^bSlope and standard error based on natural logarithm HLA-DR cells versus log₂ dioxin.**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.****Log₂ (initial dioxin)-by-covariate interaction (p≤0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-10. (Continued)

Analysis of HLA-DR Cells

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=197) (R ² =0.582)	≤18.6	404.1 (22)	467.5 (49)	380.1 (23)	-0.058 (0.050)	0.198 ^c 0.244 ^d	
	>18.6	390.1 (25)	475.8 (48)	440.3 (30)	0.033 (0.051)	0.516 ^d	
f) Maximal (n=275) (R ² =0.548)	≤18.6	430.2 (39)	453.8 (70)	356.7 (31)	-0.043 (0.035)	0.131 ^c 0.216 ^d	
	>18.6	406.1 (24)	424.2 (68)	469.2 (43)	0.033 (0.035)	0.336 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=196) (R ² =0.652)	≤18.6	415.4 (22)	464.1 (49)	404.8 (23)	-0.049 (0.047)	0.112 ^c 0.300 ^d	CSMOK (p=0.075) ALC (p=0.083) DRKYR (P=0.105)
	>18.6	383.2 (25)	455.5 (47)	463.9 (30)	0.056 (0.047)	0.240 ^d	
h) Maximal (n=274) (R ² =0.632)	≤18.6	443.2 (39)	454.4 (70)	377.2 (31)	-0.037 (0.033)	0.136 ^c 0.255 ^d	CSMOK (p<0.001) AGE*DRKYR (p=0.044)
	>18.6	430.1 (24)	414.4 (67)	457.5 (43)	0.032 (0.032)	0.329 ^d	

^aTransformed from natural logarithm scale.^bSlope and standard error based on natural logarithm HLA-DR cells versus log₂ dioxin.^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-10. (Continued)
Analysis of HLA-DR Cells (cells/mm³)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	300	422.5	All Categories		0.520
Unknown	127	433.7	Unknown vs. Background	11.2 --	0.574
Low	73	459.5	Low vs. Background	37.0 --	0.138
High	74	433.2	High vs. Background	10.7 --	0.664
Total	574		(R ² =0.360)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	300	****	All Categories		****	DXCAT*AGE (p=0.003)
Unknown	127	****	Unknown vs. Background	****	****	CSMOK (p<0.001)
Low	73	****	Low vs. Background	****	****	
High	74	****	High vs. Background	****	****	
Total	574		(R ² =0.444)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fP-value is based on difference of means on natural logarithm scale.

****Categorized current dioxin-by-covariate interaction (p≤0.01); adjusted mean, and p-value not presented.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

Under the maximal assumption, older Ranch Hands who had one drink or less per day displayed a significant positive association between HLA-DR cells and initial dioxin (Appendix Table O-1: $p=0.019$). For the other three strata combinations of age and current alcohol use, there were nonsignificant negative associations. After excluding both interactions from the model, there was a nonsignificant association between HLA-DR cells and initial dioxin (Table 16-10 [d]: $p=0.943$).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of HLA-DR cells, the interaction of current dioxin and time since tour was not significant for both the minimal and maximal assumptions (Table 16-10 [e] and [f]: $p=0.198$ and $p=0.131$).

Under both the minimal and maximal assumptions, the adjusted analysis also exhibited nonsignificant interactions between current dioxin and time (Table 16-10 [g] and [h]: $p=0.112$ and $p=0.136$, respectively).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis indicated that the HLA-DR cell means did not differ significantly among the Ranch Hand and Comparison current dioxin categories (Table 16-10 [i]: $p=0.520$).

The adjusted analysis of the HLA-DR cells contained a significant interaction between categorized current dioxin and age (Table 16-10 [j]: $p=0.003$). The interaction was explored separately for participants born in or after 1942 and those born prior to 1942 (Appendix Table O-1). For the younger participants, the overall contrast of the current dioxin categories was nonsignificant ($p=0.157$). For the older group of participants, the overall contrast of the adjusted HLA-DR cell means for the four current dioxin categories was significant ($p=0.027$) and the three contrasts of Ranch Hands versus Comparisons were at least marginally significant (unknown versus background, $p=0.052$; low versus background, $p=0.058$; high versus background, $p=0.015$).

CD4/CD8 Ratio

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

Under the minimal assumption, the unadjusted analysis of the association between the CD4/CD8 ratio and initial dioxin was not significant (Table 16-11 [a]: $p=0.230$). Under the maximal assumption, the unadjusted analysis of the association between CD4/CD8 and initial dioxin was marginally significant (Table 16-11 [b]: $p=0.074$). The mean CD4/CD8 ratios for low, medium, and high initial dioxin were 1.70, 1.91, and 1.90.

In the adjusted analysis under the minimal assumption, the association between CD4/CD8 and initial dioxin was not significant (Table 16-11 [c]: $p=0.397$). In the adjusted analysis under the maximal assumption, none of the covariates or interactions were retained in the model. Therefore, the unadjusted and adjusted results are the same for the maximal cohort (Table 16-11 [b] and [d]).

TABLE 16-11.
Analysis of CD4/CD8 Ratio

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=193) (R ² =0.635)	Low	45	1.75	0.040 (0.033)	0.230
	Medium	95	1.99		
	High	53	2.01		
b) Maximal (n=270) (R ² =0.578)	Low	64	1.70	0.042 (0.023)	0.074
	Medium	135	1.91		
	High	71	1.90		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=193) (R ² =0.649)	Low	45	1.77	0.028 (0.033)	0.397	PACKYR (p=0.061)
	Medium	95	2.01			
	High	53	1.96			
d) Maximal (n=270) (R ² =0.578)	Low	64	1.70	0.042 (0.023)	0.074	- -
	Medium	135	1.91			
	High	71	1.90			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm CD4/CD8 ratio versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-11. (Continued)

Analysis of CD4/CD8 Ratio

Ranch Hands - Log₂ (Current Dioxin) and Time - Unadjusted

Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value
		Low	Medium	High		
e) Minimal (n=193) (R ² =0.639)	≤18.6	1.84	1.97	1.92	0.075 (0.050)	0.318 ^c
		(22)	(48)	(22)		0.142 ^d
	>18.6	1.92	1.96	2.00	0.005 (0.048)	0.917 ^d
		(24)	(47)	(30)		
f) Maximal (n=270) (R ² =0.581)	≤18.6	1.75	1.88	2.18	0.062 (0.037)	0.657 ^c
		(39)	(70)	(29)		0.093 ^d
	>18.6	1.62	1.88	1.87	0.038 (0.036)	0.283 ^d
		(23)	(66)	(43)		

Ranch Hands - Log₂ (Current Dioxin) and Time - Adjusted

Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=193) (R ² =0.655)	≤18.6	1.86	1.94	1.87	0.064 (0.050)	0.237 ^c	PACKYR (p=0.048)
		(22)	(48)	(22)		0.200 ^d	
	>18.6	2.03	1.98	1.92	-0.017 (0.048)	0.725 ^d	
		(24)	(47)	(30)			
h) Maximal (n=270) (R ² =0.581)	≤18.6	1.75	1.88	2.18	0.062 (0.037)	0.657 ^c	--
		(39)	(70)	(29)		0.093 ^d	
	>18.6	1.62	1.88	1.87	0.038 (0.036)	0.283 ^d	
		(23)	(66)	(43)			

^aTransformed from natural logarithm scale.^bSlope and standard error based on natural logarithm CD4/CD8 ratio versus log₂ dioxin.^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-11. (Continued)

Analysis of CD4/CD8 Ratio

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	301	1.89	All Categories		0.196
Unknown	126	1.71	Unknown vs. Background	-0.18 --	0.068
Low	72	1.90	Low vs. Background	0.01 --	0.969
High	72	1.98	High vs. Background	0.09 --	0.548
Total	571		(R ² =0.302)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	301	1.89	All Categories		0.214	CSMOK*PACKYR (p=0.002)
Unknown	126	1.72	Unknown vs. Background	-0.17 --	0.088	
Low	72	1.91	Low vs. Background	0.02 --	0.893	
High	72	1.99	High vs. Background	0.10 --	0.477	
Total	571		(R ² =0.320)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fp-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

Under the minimal assumption, the unadjusted analysis of the CD4/CD8 ratio was not significant for the interaction of current dioxin and time since tour (Table 16-11 [e]: $p=0.318$). Thus, the association between the CD4/CD8 ratio and current dioxin did not differ significantly between the two time strata.

Under the maximal assumption, the interaction of current dioxin and time of the unadjusted model was not significant (Table 16-11 [f]: $p=0.657$) for the CD4/CD8 ratio. Although the slopes for the two time strata were not significantly different, there was a marginally significant positive association for the CD4/CD8 ratio with current dioxin among the Ranch Hands with time less than or equal to 18.6 years ($p=0.093$). For that time stratum, the average CD4/CD8 ratios for low, medium, and high current dioxin were 1.75, 1.88, and 2.18.

In the adjusted analysis of the CD4/CD8 ratio under the minimal assumption, the adjusted slopes were not significantly different between the two time strata (Table 16-11 [g]: $p=0.237$). For Ranch Hands in the maximal cohort, none of the covariates was retained in the adjusted model; thus, the unadjusted and adjusted analysis results were the same (as seen in Table 16-11 [f] and [h]).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the CD4/CD8 ratio, the overall contrast of the unadjusted CD4/CD8 means was nonsignificant (Table 16-11 [i]: $p=0.196$) for the four current dioxin categories. The individual contrast for Ranch Hands in the unknown current dioxin category versus Comparisons in the background current dioxin category was marginally significant ($p=0.068$), with the Ranch Hands having the lower mean (1.71 versus 1.89).

Similar to the results of the unadjusted analysis of the CD4/CD8 ratio, the adjusted analysis also exhibited a nonsignificant overall contrast (Table 16-11 [j]: $p=0.214$) and the unknown versus background current dioxin category contrast was marginally significant ($p=0.088$).

Laboratory Examination Variables: Quantitative Studies—TLC

TLC

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

Under both the minimal and maximal assumptions, the unadjusted analysis exhibited a nonsignificant association between TLC and initial dioxin (Table 16-12 [a] and [b]: $p=0.841$ and $p=0.679$, respectively).

For the minimal cohort, the adjusted analysis contained a significant interaction between initial dioxin and current alcohol use (Table 16-12 [c]: $p=0.018$). Investigation of this interaction within dichotomized current alcohol use strata (zero to one drink per day, over one drink per day) identified a significant negative association between TLC and initial dioxin

TABLE 16-12.
Analysis of TLC (cells/mm³)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=197) (R ² <0.001)	Low	45	2,070.9	-0.004 (0.020)	0.841
	Medium	98	2,047.0		
	High	54	1,994.6		
b) Maximal (n=275) (R ² <0.001)	Low	65	2,043.4	0.006 (0.015)	0.679
	Medium	137	2,007.8		
	High	73	2,026.4		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=196) (R ² =0.140)	Low	45	2,111.0**	-0.005 (0.020)**	0.812**	INIT*ALC (p=0.018) AGE (p=0.039) CSMOK (p=0.092) PACKYR (p=0.142) DRKYR (p=0.019)
	Medium	97	2,014.2**			
	High	54	1,999.6**			
d) Maximal (n=274) (R ² =0.116)	Low	65	2,072.4	0.001 (0.014)	0.957	CSMOK (p<0.001) AGE*DRKYR (p=0.001)
	Medium	136	2,001.7			
	High	73	1,996.4			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm TLC versus log₂ dioxin.

**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-12. (Continued)
Analysis of TLC (cells/mm³)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=197) (R ² =0.013)	≤18.6	1,996.3 (22)	2,003.9 (49)	1,934.9 (23)	-0.033 (0.031)	0.264 ^c 0.283 ^d	
	>18.6	2,095.7 (25)	2,075.1 (48)	2,100.3 (30)	0.013 (0.027)	0.636 ^d	
f) Maximal (n=275) (R ² =0.006)	≤18.6	1,974.4 (39)	2,041.5 (70)	1,871.4 (31)	-0.011 (0.022)	0.367 ^c 0.604 ^d	
	>18.6	2,010.6 (24)	2,018.7 (68)	2,157.1 (43)	0.016 (0.021)	0.446 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=196) (R ² =0.131)	≤18.6	2,052.2 (22)	2,000.2 (49)	1,905.1 (23)	-0.048 (0.030)	0.085 ^c 0.110 ^d	AGE (p=0.035) CSMOK (p=0.109) PACKYR (p=0.121)
	>18.6	2,067.1 (25)	2,036.7 (47)	2,136.1 (30)	0.019 (0.027)	0.485 ^d	DRKYR (p=0.027)
h) Maximal (n=274) (R ² =0.138)	≤18.6	2,185.3 (39)	2,227.3 (70)	2,018.7 (31)	-0.020 (0.021)	0.216 ^c 0.350 ^d	CSMOK (p<0.001) AGE*DRKYR (p=0.002) RACE*ALC (p=0.040)
	>18.6	2,273.1 (24)	2,184.1 (67)	2,324.7 (43)	0.016 (0.020)	0.442 ^d	

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm TLC versus log₂ dioxin.

^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-12. (Continued)

Analysis of TLC (cells/mm³)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	301	1,972.0	All Categories		0.817
Unknown	127	1,954.2	Unknown vs. Background	-17.8 --	0.789
Low	73	2,011.6	Low vs. Background	39.6 --	0.635
High	74	2,032.4	High vs. Background	60.4 --	0.468
Total	575		(R ² =0.002)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	300	****	All Categories		****	DXCAT*AGE (p=0.004)
Unknown	127	****	Unknown vs. Background	****	****	DXCAT*DRKYR (p=0.048)
Low	72	****	Low vs. Background	****	****	RACE (p=0.051)
High	74	****	High vs. Background	****	****	CSMOK (p<0.001)
Total	573		(R ² =0.124)			

^aTransformed from natural logarithm scale.^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.^fP-value is based on difference of means on natural logarithm scale.

****Categorized current dioxin-by-covariate interaction (p≤0.01); adjusted mean, and p-value not presented.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

for Ranch Hands who had more than one drink per day (Appendix Table O-1: $p=0.013$). For the other stratum, there was a nonsignificant positive association ($p=0.500$). Without this interaction in the model, the association between TLC and initial dioxin was not significant (Table 16-12 [c]: $p=0.812$).

Under the maximal assumption, the adjusted analysis displayed a nonsignificant association between TLC and initial dioxin (Table 16-12 [d]: $p=0.957$).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

For both the minimal and maximal assumptions, the interaction of current dioxin and time since tour was not significant in the unadjusted analysis of TLC (Table 16-12 [e] and [f]: $p=0.264$ and $p=0.367$, respectively).

Under the minimal assumption, the adjusted analysis exhibited a marginally significant interaction between current dioxin and time; thus, the associations of the two time strata (i.e., the adjusted slopes) differed marginally between the two strata (Table 16-12 [g]: $p=0.085$). Covariates retained in the adjusted model were age, current and lifetime cigarette smoking, and lifetime alcohol history. Within each time stratum, the association between TLC and current dioxin was not significant. For time less than or equal to 18.6 years, there was a nonsignificant negative association ($p=0.110$), and for time over 18.6 years, there was a nonsignificant positive association ($p=0.485$).

Under the maximal assumption, the analysis indicated that the adjusted slopes for the two time strata were not significantly different (Table 16-12 [h]: $p=0.216$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis indicated that TLC means among the Ranch Hand and Comparison current dioxin categories were not significantly different (Table 16-12 [i]: $p=0.817$).

In the adjusted analysis of TLC, there were significant interactions between categorized current dioxin and age, and between categorized current dioxin and lifetime alcohol history (Table 16-12 [j]: $p=0.004$ and $p=0.048$, respectively). To examine the interactions, age was dichotomized for participants born in or after 1942 and those born before 1942, and lifetime alcohol history was trichotomized for participants with 0 drink-years, 40 drink-years or less, and over 40 drink-years. Contrasts of Ranch Hands and Comparisons were performed for each of the six strata combinations of age and lifetime alcohol history. The analysis using nondrinking participants born in or after 1942 was based on small sample sizes. For the two younger Ranch Hands who did not drink, the contrast of the high current dioxin category versus the background current dioxin category was significant (Appendix Table O-1: $p=0.021$) with the Comparisons having the higher adjusted TLC mean. For younger Ranch Hands with 40 drink-years or less of alcohol history, the unknown current dioxin category differed significantly from the background current dioxin category ($p=0.048$) with the Comparisons again having the higher adjusted TLC mean. For older Ranch Hands with 40 drink-years or less of lifetime alcohol history, marginally significant differences were found for the unknown versus background contrast ($p=0.065$) and the high versus background contrast

($p=0.086$). For these contrasts, the adjusted TLC mean of the Ranch Hands exceeded that of the background Comparisons.

Laboratory Examination Data: Functional Stimulation Tests

Unstimulated PHA Response

The analyses of the unstimulated PHA responses were based on two-factor repeated measures models containing a dioxin measure, mitogen harvest day, and the dioxin-by-harvest day interaction. The unadjusted models were expanded to include the batch-to-batch and blood draw day-to-day covariates. The adjusted models also included these covariates, as well as any covariates that were retained from the stepwise modeling procedure. For the minimal and maximal assumptions, the initial dioxin-by-harvest day interaction was not significant for the model 1 analyses (minimal: $p=0.792$; maximal: $p=0.441$). Similarly, the current dioxin-by-time-by-harvest day interaction was not significant under both assumptions for the model 2 analyses (minimal: $p=0.173$; maximal: $p=0.758$). Lastly, the categorized current dioxin-by-harvest day interaction was nonsignificant for the model 3 analyses ($p=0.529$). Therefore, main effect associations between unstimulated PHA response and dioxin were evaluated for all models across harvest day.

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

For both the minimal and maximal assumptions, the unadjusted analysis of the unstimulated PHA response was not significant for an association with initial dioxin (Table 16-13 [a] and [b]: $p=0.604$ and $p=0.174$, respectively).

For both the minimal and maximal assumptions, the adjusted analysis of the unstimulated PHA response also was nonsignificant for an association with initial dioxin (Table 16-13 [c] and [d]: $p=0.464$ and $p=0.459$, respectively).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

For both assumptions, the unadjusted analysis indicated that the associations between unstimulated PHA and current dioxin did not differ significantly between the two time since tour strata (Table 16-13 [e] and [f]: $p=0.884$ and $p=0.878$, respectively).

Similarly, the adjusted analyses exhibited nonsignificant interactions between current dioxin and time for both cohorts (Table 16-13 [g] and [h]: $p=0.553$ and $p=0.884$, respectively).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis of unstimulated PHA response indicated that the mean levels for the Ranch Hands and Comparisons did not differ significantly (Table 16-13 [i]: $p=0.679$).

The adjusted analysis of the unstimulated PHA response also indicated that the overall contrast of the adjusted means for Ranch Hands and Comparisons did not differ significantly (Table 16-13 [j]: $p=0.765$).

TABLE 16-13.
Analysis of Unstimulated PHA Response (cpm)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=193) (R ² =0.660)	Low	44	2,083	0.021 (0.040)	0.604
	Medium	96	2,227		
	High	53	2,116		
b) Maximal (n=268) (R ² =0.613)	Low	63	1,871	0.035 (0.025)	0.174
	Medium	134	2,014		
	High	71	2,146		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=192) (R ² =0.737)	Low	44	2,142	0.027 (0.037)	0.464	CSMOK (p=0.015) PACKYR (p=0.046) ALC*DRKYR (p=0.003)
	Medium	95	2,154			
	High	53	2,186			
d) Maximal (n=267) (R ² =0.670)	Low	63	2,142	0.019 (0.025)	0.459	AGE (p=0.085) CSMOK (p=0.143) PACKYR (p=0.085) ALC*DRKYR (p=0.010)
	Medium	133	2,025			
	High	71	2,115			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm unstimulated PHA response versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-13. (Continued)
Analysis of Unstimulated PHA Response (cpm)

Ranch Hands - Log₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=193) (R ² =0.663)	≤18.6	1,946 (21)	2,418 (48)	2,215 (23)	0.038 (0.059)	0.884 ^c 0.514 ^d	
	>18.6	2,105 (25)	2,128 (47)	2,027 (29)	0.026 (0.059)	0.656 ^d	
f) Maximal (n=268) (R ² =0.615)	≤18.6	1,810 (39)	2,118 (67)	2,098 (31)	0.048 (0.040)	0.878 ^c 0.228 ^d	
	>18.6	1,945 (22)	1,959 (67)	2,119 (42)	0.039 (0.039)	0.322 ^d	
Ranch Hands - Log₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=192) (R ² =0.734)	≤18.6	2,276 (21)	2,343 (48)	2,200 (23)	-0.0004 (0.057)	0.553 ^c 0.994 ^d	AGE (p=0.097) ALC*DRKYR (p=0.002)
	>18.6	2,027 (25)	2,039 (46)	2,030 (29)	0.045 (0.056)	0.416 ^d	
h) Maximal (n=267) (R ² =0.672)	≤18.6	1,848 (39)	2,130 (67)	2,046 (31)	0.032 (0.039)	0.884 ^c 0.414 ^d	AGE (p=0.116) CSMOK (p=0.138) PACKYR (p=0.098)
	>18.6	2,096 (22)	1,921 (66)	2,039 (42)	0.024 (0.039)	0.538 ^d	ALC*DRKYR (p=0.012)

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm unstimulated PHA response versus log₂ dioxin.

^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-13. (Continued)
Analysis of Unstimulated PHA Response (cpm)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	297	2,003	All Categories		0.679
Unknown	123	1,962	Unknown vs. Background	-41 --	0.689
Low	71	2,129	Low vs. Background	126 --	0.332
High	73	2,064	High vs. Background	61 --	0.640
Total	564		(R ² =0.541)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	296	2,144	All Categories		0.765	AGE (p<0.001) RACE (p=0.074) DRKYR (p=0.053)
Unknown	123	2,137	Unknown vs. Background	-7 --	0.951	
Low	70	2,284	Low vs. Background	140 --	0.304	
High	73	2,168	High vs. Background	24 --	0.860	
Total	562		(R ² =0.565)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fP-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

PHA Net Response

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

To investigate the effects of initial dioxin on PHA net response, the analyses of the six PHA net responses (for 2 mitogen harvest days at each of 3 mitogen concentrations) were based on three-factor repeated measures models containing initial dioxin, mitogen harvest day, mitogen concentration, associated two-factor interactions, and a three-factor interaction. The unadjusted models were expanded to include the batch-to-batch and blood draw day-to-day covariates. The adjusted models also included these covariates, as well as any covariates that were retained from the stepwise modeling procedure. From the repeated measures analysis, nonsignificant interactions were found for the initial dioxin-by-harvest day effect (minimal: $p=0.361$; maximal: $p=0.465$) and the initial dioxin-by-harvest day-by-mitogen concentration effect (minimal: $p=0.324$; maximal: $p=0.282$). For both cohorts, the initial dioxin-by-mitogen concentration interaction was significant for the initial dioxin analyses (minimal: $p=0.011$; maximal: $p=0.001$). Because of those significant interactions, unadjusted and adjusted analyses were performed separately for each mitogen concentration.

Mitogen Concentration 1. The unadjusted analyses did not exhibit a significant association between PHA net response and initial dioxin under both assumptions (Table 16-14 [a1] and [b1]: minimal: $p=0.418$; maximal: $p=0.950$).

For mitogen concentration 1, the adjusted analysis had a significant initial dioxin-by-lifetime alcohol history interaction for the minimal cohort and a significant interaction between initial dioxin and lifetime cigarette smoking history for the maximal cohort (Table 16-14 [c1] and [d1]: $p<0.001$ and $p=0.014$, respectively). To investigate the former interaction, separate analyses were performed under the minimal assumption for Ranch Hands with lifetime alcohol history values of 0 drink-years, up to 40 drink-years, and over 40 drink-years. For the nondrinker stratum, there was a significant negative association between PHA net response and initial dioxin (Appendix Table O-1: $p=0.014$). For the over 40 drink-year stratum, there was a significant positive association between PHA net response and initial dioxin ($p=0.015$). The other drink-year stratum exhibited a nonsignificant positive association between PHA net response and initial dioxin ($p=0.920$). Separate analyses were also performed, under the maximal assumption, for Ranch Hands with lifetime cigarette smoking history values of 0 pack-years, up to 10 pack-years, and over 10 pack-years. None of the adjusted relationships between PHA net response and initial dioxin was significant within the three smoking strata ($p>0.25$ for all strata). Under the maximal assumption, a secondary analysis was performed without the interaction of initial dioxin and lifetime cigarette smoking history in the model. For that model, the association between PHA net response and initial dioxin was not significant (Table 16-14 [d1]: $p=0.742$).

Mitogen Concentration 2. The unadjusted analyses of the PHA net response exhibited significant positive associations with initial dioxin under the minimal and maximal assumptions (Table 16-14 [a2] and [b2]: $p=0.016$ and $p=0.008$). Under the minimal assumption, the mean PHA net responses for low, medium, and high initial dioxin were 153,870 cpm, 182,316 cpm, and 190,835 cpm. Under the maximal assumption, the corresponding mean PHA net responses were 170,046 cpm, 162,750 cpm, and 189,735 cpm.

TABLE 16-14.
Analysis of PHA Net Response (cpm)
(Concentration 1)

Ranch Hands - Log₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean	Slope (Std. Error)^a	p-Value
a1) Minimal (n=192) (R ² =0.784)	Low	44	114,027	2,988 (3,671)	0.418
	Medium	96	135,873		
	High	52	128,374		
b1) Maximal (n=265) (R ² =0.786)	Low	61	130,601	137 (2,202)	0.950
	Medium	134	124,212		
	High	70	128,932		

Ranch Hands - Log₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error)^a	p-Value	Covariate Remarks
c1) Minimal (n=191) (R ² =0.857)	Low	44	****	****	****	INIT*DRKYR (p<0.001) AGE*DRKYR (p=0.005) CSMOK*DRKYR (p=0.001) PACKYR*DRKYR (p<0.001)
	Medium	95	****			
	High	52	****			
d1) Maximal (n=264) (R ² =0.819)	Low	61	129,143**	-728 (2,205)**	0.742**	INIT*PACKYR (p=0.014) AGE (p=0.017) CSMOK (p=0.045) DRKYR*PACKYR (p=0.001)
	Medium	133	125,792**			
	High	70	126,836**			

^aSlope and standard error based on PHA net response over concentration 1 versus log₂ dioxin.

**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

****Log₂ (initial dioxin)-by-covariate interaction (p≤0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-14. (Continued)
Analysis of PHA Net Response (cpm)
(Concentration 2)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean	Slope (Std. Error) ^a	p-Value
a2) Minimal (n=192) (R ² =0.847)	Low	44	153,870	8,529 (3,475)	0.016
	Medium	96	182,316		
	High	52	190,835		
b2) Maximal (n=265) (R ² =0.775)	Low	61	170,046	6,768 (2,528)	0.008
	Medium	134	162,750		
	High	70	189,735		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
c2) Minimal (n=192) (R ² =0.854)	Low	44	157,522	6,163 (3,595)	0.090	AGE (p=0.040)
	Medium	96	181,755			
	High	52	187,901			
d2) Maximal (n=265) (R ² =0.793)	Low	61	170,972	4,479 (2,525)	0.078	AGE (p=0.001)
	Medium	134	164,724			
	High	70	184,295			

^aSlope and standard error based on PHA net response for concentration 2 versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-14. (Continued)
Analysis of PHA Net Response (cpm)
(Concentration 3)

Ranch Hands - Log₂ (Initial Dioxin) - Unadjusted

Assumption	Initial Dioxin	n	Mean	Slope (Std. Error) ^a	p-Value
a3) Minimal (n=192) (R ² =0.823)	Low	44	124,733	4,598 (3,249)	0.161
	Medium	96	148,991		
	High	52	151,319		
b3) Maximal (n=265) (R ² =0.694)	Low	61	140,798	3,352 (2,600)	0.199
	Medium	134	132,626		
	High	70	147,992		

Ranch Hands - Log₂ (Initial Dioxin) - Adjusted

Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
c3) Minimal (n=192) (R ² =0.828)	Low	44	125,197	3,760 (3,263)	0.252	PACKYR (p=0.115)
	Medium	96	149,569			
	High	52	149,262			
d3) Maximal (n=264) (R ² =0.726)	Low	61	162,992**	1,824 (2,636)**	0.490**	INIT*ALC (p=0.048) AGE (p=0.011) RACE (p=0.025)
	Medium	133	156,117**			
	High	70	165,787**			

^aSlope and standard error based on PHA net response for concentration 3 versus log₂ dioxin.

**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-14. (Continued)

**Analysis of PHA Net Response (cpm)
(Across Day and Concentration)**

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean	Slope (Std. Error) ^a	p-Value
a4) Minimal (n=192) (R ² =0.839)	Low	44	130,877	5,372 (3,010)	0.078
	Medium	96	155,727		
	High	52	156,842		
b4) Maximal (n=265) (R ² =0.785)	Low	61	147,148	3,419 (2,096)	0.105
	Medium	134	139,863		
	High	70	155,553		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
c4) Minimal (n=191) (R ² =0.873)	Low	44	133,518**	3,093 (3130)**	0.326**	INIT*PACKYR (p=0.014) AGE (p=0.046) CSMOK (p=0.060) DRKYR*PACKYR (p=0.003)
	Medium	95	155,345**			
	High	52	152,978**			

^aSlope and standard error based on PHA net response across day and concentration versus log₂ dioxin.

**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-14. (Continued)
Analysis of PHA Net Response (cpm)

Ranch Hands - Log₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean/(n) Current Dioxin			Slope (Std. Error) ^a	p-Value	
		Low	Medium	High			
e) Minimal (n=192) (R ² =0.848)	≤18.6	133,816 (21)	152,504 (48)	176,224 (22)	12,371 (4,364)	0.057 ^b 0.006 ^c	
	>18.6	133,168 (25)	157,289 (47)	147,659 (29)	613 (4,297)	0.887 ^c	
f) Maximal (n=265) (R ² =0.791)	≤18.6	151,679 (38)	141,822 (67)	168,037 (30)	5,291 (3,278)	0.891 ^b 0.109 ^c	
	>18.6	129,615 (21)	139,635 (67)	149,292 (42)	4,641 (3,215)	0.151 ^c	
Ranch Hands - Log₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean/(n) Current Dioxin			Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=191) (R ² =0.884)	≤18.6	139,317** (21)	148,268** (48)	175,433** (22)	10,167 (4,693)**	0.069** ^b 0.033** ^c	CURR*TIME*DRKYR (p=0.017)
	>18.6	134,282** (25)	160,395** (46)	143,333** (29)	-959 (4,348)**	0.826** ^c	AGE*DRKYR (p=0.002) CSMOK*DRKYR (p=0.038)
h) Maximal (n=264) (R ² =0.815)	≤18.6	153,521 (38)	143,054 (67)	163,151 (30)	3,705 (3,235)	0.976 ^b 0.254 ^c	AGE (p=0.008) PACKYR*DRKYR (p=0.011)
	>18.6	129,873 (21)	140,630 (66)	148,487 (42)	3,565 (3,182)	0.265 ^c	

^aSlope and standard error based on PHA net response versus log₂ dioxin.

^bTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^cTest of significance for slope different from 0 (current dioxin continuous, time categorized).

**Log₂ (current dioxin)-by-time-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-14. (Continued)
Analysis of PHA Net Response (cpm)
(Concentration 1)

ii) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.)	p-Value
Background	297	126,096	All Categories		0.810
Unknown	121	129,147	Unknown vs. Background	3,051 (-7,006, 13,108)	0.552
Low	71	130,539	Low vs. Background	4,444 (-7,650, 16,538)	0.472
High	72	130,671	High vs. Background	4,575 (-7,840, 16,990)	0.471
Total	561		(R ² =0.668)		

j1) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	297	125,477	All Categories		0.745	AGE (p<0.001) CSMOK (p=0.063)
Unknown	121	130,104	Unknown vs. Background	4,627 (-5,222, 14,477)	0.358	
Low	71	130,318	Low vs. Background	4,842 (-6,988, 16,671)	0.423	
High	72	127,162	High vs. Background	1,686 (-10,507, 13,879)	0.787	
Total	561		(R ² =0.685)			

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

TABLE 16-14. (Continued)
Analysis of PHA Net Response (cpm)
(Concentration 2)

i2) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.)	p-Value
Background	297	166,313	All Categories		0.042
Unknown	121	160,792	Unknown vs. Background	-5,521 (-15,976, 4,933)	0.301
Low	71	171,010	Low vs. Background	4,696 (-7,876, 17,269)	0.465
High	72	181,128	High vs. Background	14,815 (1,909, 27,721)	0.025
Total	561		(R ² =0.670)		

j2) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	297	165,397	All Categories		0.216	AGE (p<0.001) SMOK (p=0.040)
Unknown	121	162,332	Unknown vs. Background	-3,065 (-12,962, 6,832)	0.544	
Low	71	170,936	Low vs. Background	5,539 (-6,348, 17,426)	0.362	
High	72	175,692	High vs. Background	10,295 (-1,957, 22,547)	0.100	
Total	561		(R ² =0.707)			

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

TABLE 16-14. (Continued)
Analysis of PHA Net Response (cpm)
(Concentration 3)

i3) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.)	p-Value
Background	297	131,602	All Categories		0.223
Unknown	121	129,248	Unknown vs. Background	-2,354 (-12,762, 8,054)	0.658
Low	71	141,174	Low vs. Background	9,572 (-2,945, 22,089)	0.135
High	72	140,193	High vs. Background	8,591 (-4,258, 21,441)	0.191
Total	561		(R ² =0.577)		

j3) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	296	****	All Categories		****	DXCAT*ALC (p=0.004)
Unknown	121	****	Unknown vs. Background	****	****	AGE (p<0.001)
Low	70	****	Low vs. Background	****	****	RACE*DRKYR
High	72	****	High vs. Background	****	****	(p=0.012)
Total	559		(R ² =0.627)			

****Categorized current dioxin-by-covariate interaction (p≤0.01); adjusted mean, confidence interval, and p-value not presented.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

TABLE 16-14. (Continued)
Analysis of PHA Net Response (cpm)
Across Day and Concentration

i4) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.)	p-Value
Background	297	141,337	All Categories		0.221
Unknown	121	139,729	Unknown vs. Background	-1,608 (-10,483, 7,267)	0.723
Low	71	147,574	Low vs. Background	6,237 (-4,436, 16,910)	0.253
High	72	150,664	High vs. Background	9,327 (-1,629, 20,283)	0.096
Total	561		(R ² =0.678)		

j4) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	297	146,409	All Categories		0.457	AGE (p<0.001) RACE (p=0.104) CSMOK (p=0.124)
Unknown	121	146,610	Unknown vs. Background	201 (-8,247, 8,648)	0.963	
Low	71	153,318	Low vs. Background	6,909 (-3,235, 17,053)	0.183	
High	72	152,015	High vs. Background	5,606 (-4,850, 16,062)	0.294	
Total	561		(R ² =0.712)			

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

For mitogen concentration 2, the adjusted analyses of the PHA net response exhibited marginally significant associations with initial dioxin for the minimal and maximal assumptions (Table 16-14 [c2] and [d2]: $p=0.090$ and $p=0.078$). For both cohorts, age was the only covariate retained from the stepwise modeling strategy.

Mitogen Concentration 3. The unadjusted analyses of the PHA net response displayed a nonsignificant association with initial dioxin for both the minimal and maximal assumptions (Table 16-14 [a3] and [b3]: $p=0.161$ and $p=0.199$).

For mitogen concentration 3, the adjusted analysis based on the minimal assumption was not significant for an association with initial dioxin (Table 16-14 [c3]: $p=0.252$). Under the maximal assumption, there was a significant interaction between initial dioxin and current alcohol use (Table 16-14 [d3]: $p=0.048$). To investigate this interaction, analyses were performed for Ranch Hands with current alcohol use values of zero to one drink per day and more than one drink per day. Within these individual drinking strata, the associations between PHA net response and initial dioxin were not significant (Appendix Table O-1). Under the maximal assumption, a secondary model was used that did not include the interaction between initial dioxin and current alcohol use. For that model, the association between PHA net response and initial dioxin was not significant (Table 16-14 [d3]: $p=0.490$).

Across Mitogen Harvest Day and Mitogen Concentration. As noted in the introduction to the analysis of all six PHA net response variables, there was a significant interaction between initial dioxin and mitogen concentration for both assumptions (minimal: $p=0.011$; maximal: $p=0.001$). Because the p -value for the interaction of the minimal cohort was greater than 0.01, a secondary model was used that did not assume it was necessary to evaluate the association of PHA net response and initial dioxin for each individual mitogen concentration level. Unadjusted analyses were performed under both assumptions and an adjusted analysis was performed under the minimal assumption. Because the interaction of initial dioxin and mitogen concentration was highly significant ($p=0.001$), no adjusted analysis across mitogen harvest day and mitogen concentration was pursued under the maximal assumption.

Under the minimal assumption, the unadjusted analysis indicated that there was a positive association, which was marginally significant, between PHA net response and initial dioxin across mitogen harvest day and mitogen concentration (Table 16-14 [a4]: $p=0.078$). The mean PHA net response at the low, medium, and high initial dioxin levels were 130,877 cpm, 155,727 cpm, and 156,842 cpm. Under the maximal assumption, the unadjusted analysis displayed a nonsignificant association (Table 16-14 [b4]: $p=0.105$).

Under the minimal assumption, the adjusted analysis exhibited a significant initial dioxin-by-lifetime cigarette smoking history interaction (Table 16-14 [c4]: $p=0.014$). This interaction was explored within each of the following three lifetime cigarette smoking history strata: 0 pack-years, up to 10 pack-years, and over 10 pack-years. For Ranch Hands with a value above 10 pack-years, there was a positive association of borderline significance between PHA net response and initial dioxin (Appendix Table O-1: $p=0.075$). For the nonsmokers, there was a nonsignificant negative association ($p=0.596$), and for the moderate

smokers, there was a nonsignificant positive association ($p=0.426$). A secondary model without the interaction displayed a nonsignificant association between PHA net response and initial dioxin ($p=0.326$).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

To investigate the effects of current dioxin and time since tour on PHA net response, the analyses of the six PHA net responses were based on four-factor repeated measures models containing current dioxin, time, mitogen harvest day, mitogen concentration, associated two-factor and three-factor interactions, and a four-factor interaction. The unadjusted models were again expanded to include the batch-to-batch and blood draw day-to-day covariates. The adjusted models also included these covariates, as well as any covariates that were retained from the stepwise modeling procedure. Under both assumptions, the three-factor interactions of mitogen concentration-by-current dioxin-by-time and harvest day-by-current dioxin-by-time were not significant (minimal: $p=0.759$ and $p=0.871$, respectively; maximal: $p=0.916$ and $p=0.587$, respectively), as well as the four-factor interaction of harvest day-by-mitogen concentration-by-current dioxin-by-time (minimal: $p=0.745$; maximal: $p=0.744$).

In the unadjusted analysis of the PHA net response under the minimal assumption, there was a marginally significant interaction of current dioxin and time (Table 16-14 [e]: $p=0.057$) indicating that the associations with current dioxin differed between time strata. For time less than or equal to 18.6 years, there was a significant positive association between PHA net response and current dioxin ($p=0.006$). Within that time strata, the mean PHA net responses for low, medium, and high current dioxin were 133,816 cpm, 152,504 cpm, and 176,224 cpm. For time over 18.6 years, there was a nonsignificant positive association with current dioxin ($p=0.887$).

The unadjusted analysis under the maximal assumption did not exhibit a significant current dioxin-by-time interaction (Table 16-14 [f]: $p=0.891$).

Under the minimal assumption, the adjusted analysis contained a significant interaction between current dioxin, time, and lifetime alcohol history (Table 16-14 [g]: $p=0.017$). The interaction was investigated within the following lifetime alcohol history strata: 0 to 40 drink-years and over 40 drink-years (Appendix Table O-1). For the former alcohol history stratum, the association between PHA net response and current dioxin did not differ significantly between time strata ($p=0.485$). For the latter lifetime alcohol history stratum, the interaction of current dioxin and time was significant ($p=0.006$); there was a significant positive association between PHA net response and current dioxin for Ranch Hands with time since tour less than or equal to 18.6 years ($p=0.002$). For that time stratum, the adjusted PHA net response means for low, medium, and high current dioxin were 68,227 cpm, 139,403 cpm, and 196,600 cpm. For time over 18.6 years, the negative association was nonsignificant ($p=0.530$). Because the p-value for the significant interaction of current dioxin, time, and lifetime alcohol history was greater than 0.01, a secondary analysis was performed based on a model without the interaction. For that adjusted analysis, the interaction between current dioxin and time was marginally significant (Table 16-14 [g]: $p=0.069$). For time less than or equal to 18.6 years, there was a significant positive association with current dioxin ($p=0.033$) and for time over 18.6 years there was a nonsignificant negative association ($p=0.826$).

The adjusted analysis of PHA net response under the maximal assumption exhibited a nonsignificant interaction between current dioxin and time (Table 16-14 [h]: $p=0.976$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

To investigate the effects of current dioxin in Ranch Hands and Comparisons on PHA net response, the analyses of the six PHA net responses (for 2 mitogen harvest days at each of 3 mitogen concentrations) were based on three-factor repeated measures models containing categorized current dioxin, mitogen harvest day, mitogen concentration, associated two-factor interactions, and a three-factor interaction. The unadjusted models were expanded to include the batch-to-batch and blood draw day-to-day covariates. The adjusted models also included these covariates, as well as any covariates that were retained from the stepwise modeling procedure. From the repeated measures analysis, nonsignificant interactions were found for the categorized current dioxin-by-harvest day effect ($p=0.979$) and the categorized current dioxin-by-harvest day-by-mitogen concentration effect ($p=0.429$). However, the categorized current dioxin-by-mitogen concentration interaction was significant for this analysis ($p=0.010$). Because of the significant interaction, unadjusted and adjusted analyses were performed for each mitogen concentration.

Mitogen Concentration 1. The unadjusted analysis of the PHA net responses determined at concentration 1 indicated that the unadjusted means of the four current dioxin categories were not significantly different (Table 16-14 [i1]: $p=0.810$). Similarly, the adjusted analysis also indicated that the adjusted means for the Ranch Hands and Comparisons did not differ significantly (Table 16-14 [j1]: $p=0.745$).

Mitogen Concentration 2. The unadjusted analysis of PHA net responses determined at concentration 2 displayed a significant overall contrast among the Ranch Hand and Comparison current dioxin categories (Table 16-14 [i2]: $p=0.042$). The unadjusted PHA net response means were 166,313 cpm, 160,792 cpm, 171,010 cpm, and 181,128 cpm for the background, unknown, low, and high current dioxin categories. The unadjusted mean for Ranch Hands with high current dioxin was significantly greater than that of the Comparisons with background levels ($p=0.025$). After adjusting for age and current cigarette smoking, the analysis indicated that the overall contrast of the four current dioxin categories was nonsignificant (Table 16-14 [j2]: $p=0.216$). The contrast between the high versus background current dioxin categories was found to be marginally significant ($p=0.100$) with the high category having a larger PHA net response mean than the background category.

Mitogen Concentration 3. The unadjusted analysis of the PHA net responses determined at concentration 3 indicated that the means of the four current dioxin categories were not significantly different (Table 16-14 [i3]: $p=0.223$). The adjusted analysis of the PHA net responses exhibited a significant interaction between current alcohol use and the current dioxin categories (Table 16-14 [j3]: $p=0.004$). The interaction was examined for participants having zero to one drink per day, and for participants having more than one drink per day. For the lighter drinking participants, the overall contrast of the adjusted means of the PHA net responses determined at concentration 3 was nonsignificant (Appendix Table O-1: $p=0.137$). For the more frequent drinkers, the overall contrast was also found to be nonsignificant ($p=0.164$); however, the contrast between Ranch Hands in the unknown current dioxin category and Comparisons in the background current dioxin category was significant ($p=0.030$).

Across Mitogen Harvest Day and Mitogen Concentration. As noted in the introduction to the categorized current dioxin analysis, there was a significant interaction between categorized current dioxin and mitogen concentration ($p=0.010$). Because the p -value for the interaction fell within the interval 0.01 to 0.05, a secondary model was used that did not assume it was necessary to evaluate the association of PHA net response and categorized current dioxin at each individual mitogen concentration. Unadjusted analyses and adjusted analyses were performed across mitogen harvest day and mitogen concentration. In the unadjusted analysis of the means of the PHA net responses over mitogen harvest day and mitogen concentration, the overall contrast of the current dioxin categories was nonsignificant (Table 16-14 [j4]: $p=0.221$). The contrast of Ranch Hands in the high current dioxin category versus Comparisons in the background current dioxin category was marginally significant ($p=0.096$) with the Ranch Hands having the higher PHA mean. The corresponding adjusted analysis exhibited a nonsignificant overall contrast (Table 16-14 [j4]: $p=0.457$).

Maximum of Day and Concentration Level PHA Net Response

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of the maximum PHA net response (i.e., the maximum response of the six PHA net responses from 3 mitogen concentration levels and 2 mitogen harvest days), both cohorts exhibited a significant positive association with initial dioxin (Table 16-15 [a] and [b]: $p=0.005$ and $p=0.009$, respectively). Under the minimal assumption, the unadjusted means of maximum PHA net response were 184,480 cpm, 210,574 cpm, and 228,148 cpm for the low, medium, and high initial dioxin categories. Under the maximal assumption, the corresponding unadjusted means for maximum PHA net response were 205,096 cpm, 191,498 cpm, and 221,125 cpm.

In the adjusted analysis of maximum PHA net response, both the minimal and maximal cohorts also displayed a positive association with initial dioxin. However, the associations were only marginally significant (Table 16-15 [c] and [d]: $p=0.054$ and $p=0.072$, respectively). For both adjusted analyses, age and lifetime cigarette smoking history were covariates retained in the adjusted models.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of maximum PHA net response, the interaction of current dioxin and time since tour was not significant under both the minimal and maximal assumptions (Table 16-15 [e] and [f]: $p=0.145$ and $p=0.662$). However, for the minimal cohort, there was a significant positive association between maximum PHA net response and current dioxin for time less than or equal to 18.6 years ($p=0.002$). The unadjusted means for this time stratum for low, medium, and high current dioxin were 190,138 cpm, 205,695 cpm, and 245,112 cpm. For the maximal cohort, both time strata contained significant positive associations (time ≤ 18.6 years, $p=0.049$; time > 18.6 years, $p=0.008$). For time less than or equal to 18.6 years, the unadjusted means for low, medium, and high current dioxin were 210,389 cpm, 193,570 cpm, and 238,122 cpm. For time over 18.6 years, the corresponding unadjusted means were 180,289 cpm, 190,555 cpm, and 215,787 cpm.

In the adjusted analysis of the maximum PHA net response under the minimal assumption, the interaction of current dioxin and time was not significant (Table 16-15 [g]: $p=0.136$); however, similar to the corresponding unadjusted analysis, there was a significant

TABLE 16-15.

Analysis of Maximum PHA Net Response (cpm)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean	Slope (Std. Error) ^a	p-Value
a) Minimal (n=192) (R ² =0.861)	Low	44	184,480	10,112 (3,490)	0.005
	Medium	96	210,574		
	High	52	228,148		
b) Maximal (n=265) (R ² =0.804)	Low	61	205,096	6,606 (2,499)	0.009
	Medium	134	191,498		
	High	70	221,125		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
c) Minimal (n=192) (R ² =0.873)	Low	44	188,163	6,990 (3,574)	0.054	AGE (p=0.070) PACKYR (p=0.070)
	Medium	96	210,808			
	High	52	223,115			
d) Maximal (n=265) (R ² =0.823)	Low	61	204,191	4,501 (2,483)	0.072	AGE (p=0.004) PACKYR (p=0.072)
	Medium	134	193,964			
	High	70	215,003			

^aSlope and standard error based on maximum PHA net response versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-15. (Continued)
Analysis of Maximum PHA Net Response (cpm)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean/(n) Current Dioxin			Slope (Std. Error) ^a	p-Value	
		Low	Medium	High			
e) Minimal (n=192) (R ² =0.867)	≤18.6	190,138 (21)	205,695 (48)	245,112 (22)	16,692 (5,095)	0.145 ^b 0.002 ^c	
	>18.6	186,440 (25)	212,728 (47)	223,015 (29)	6,241 (5,018)	0.217 ^c	
f) Maximal (n=265) (R ² =0.812)	≤18.6	210,389 (38)	193,570 (67)	238,122 (30)	7,706 (3,880)	0.662 ^b 0.049 ^c	
	>18.6	180,289 (21)	190,555 (67)	215,787 (42)	10,170 (3,805)	0.008 ^c	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean/(n) Current Dioxin			Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=192) (R ² =0.877)	≤18.6	194,871 (21)	203,365 (48)	238,874 (22)	13,263 (5,213)	0.136 ^b 0.013 ^c	AGE (p=0.123) PACKYR (p=0.062)
	>18.6	193,326 (25)	213,510 (47)	216,867 (29)	2,760 (5,041)	0.585 ^c	
h) Maximal (n=265) (R ² =0.827)	≤18.6	210,758 (38)	194,477 (67)	229,611 (30)	5,179 (3,853)	0.685 ^b 0.181 ^c	AGE (p=0.008) PACKYR (p=0.137)
	>18.6	182,766 (21)	193,442 (67)	213,111 (42)	7,398 (3,773)	0.052 ^c	

^aSlope and standard error based on maximum PHA net response versus log₂ dioxin.

^bTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^cTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-15. (Continued)
Analysis of Maximum PHA Net Response (cpm)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.)	p-Value
Background	297	200,475	All Categories		0.037
Unknown	121	194,152	Unknown vs. Background	-6,323 (-17,188, 4,541)	0.255
Low	71	201,590	Low vs. Background	1,115 (-11,951, 14,180)	0.867
High	72	216,159	High vs. Background	15,684 (2,272, 29,097)	0.022
Total	561		(R ² =0.699)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	297	199,500	All Categories		0.221	AGE (p<0.001) CSMOK (p=0.005)
Unknown	121	195,650	Unknown vs. Background	-3,850 (-14,177, 6,477)	0.465	
Low	71	201,223	Low vs. Background	1,723 (-10,681, 14,126)	0.786	
High	72	210,652	High vs. Background	11,152 (-1,632, 23,936)	0.088	
Total	561		(R ² =0.730)			

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

positive association between maximum PHA net response and current dioxin for time less than or equal to 18.6 years ($p=0.013$). In the adjusted analysis of the maximal cohort, the current dioxin-by-time interaction was not significant (Table 16-15 [h]: $p=0.685$). For this cohort, Ranch Hands with more than 18.6 years since their tour exhibited a marginally significant positive association ($p=0.052$). For both adjusted models, age and lifetime cigarette smoking history were covariates retained in the model.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis of the maximum PHA net response indicated that the average maximum PHA net response differed significantly among current dioxin categories in Ranch Hands and Comparisons (Table 16-15 [i]: $p=0.037$). The averages for maximum PHA net response within the background, unknown, low, and high categories were 200,475 cpm, 194,152 cpm, 201,590 cpm, and 216,159 cpm. The contrast of Ranch Hands in the high current dioxin category versus Comparisons in the background current dioxin category was significant ($p=0.022$).

After adjusting for the covariates of age and current cigarette smoking, the adjusted analysis of the maximum PHA net response no longer indicated there was a significant difference among the four categories (Table 16-15 [j]: $p=0.221$). The covariate adjustment also affected the high versus background contrast in that it was now marginally significant ($p=0.088$).

Unstimulated MLC Response

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of the unstimulated MLC response, the association with initial dioxin was not significant under the minimal assumption (Table 16-16 [a]: $p=0.238$). Under the maximal assumption, the association of unstimulated MLC response and initial dioxin was marginally significant (Table 16-16 [b]: $p=0.069$). The unadjusted means for unstimulated MLC response were 3,668 cpm, 3,887 cpm, and 4,618 cpm for the low, medium, and high initial dioxin categories under the maximal assumption.

In the adjusted analysis of unstimulated MLC, both the minimal and maximal cohorts exhibited nonsignificant associations between unstimulated MLC and initial dioxin (Table 16-16 [c] and [d]: $p=0.850$ and $p=0.388$, respectively). Age, race, and the interaction of the alcohol covariates were retained in the adjusted model under the maximal assumption. Age and the cited interaction were retained under the minimal assumption.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of unstimulated MLC response, the interaction of current dioxin and time since tour was not significant under the minimal assumption (Table 16-16 [e]: $p=0.881$).

The current dioxin-by-time interaction also was not significant in the unadjusted analysis under the maximal assumption (Table 16-16 [f]: $p=0.621$); however, for Ranch Hands with time over 18.6 years, there was a marginally significant positive association between unstimulated MLC response and current dioxin ($p=0.075$).

TABLE 16-16.
Analysis of Unstimulated MLC Response (cpm)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=193) (R ² =0.742)	Low	45	3,923	0.065 (0.055)	0.238
	Medium	97	4,431		
	High	51	5,014		
b) Maximal (n=269) (R ² =0.645)	Low	63	3,668	0.074 (0.040)	0.069
	Medium	137	3,887		
	High	69	4,618		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=192) (R ² =0.788)	Low	45	4,494	0.010 (0.055)	0.850	AGE (p=0.005) ALC*DRKYR (p=0.022)
	Medium	96	4,148			
	High	51	4,716			
d) Maximal (n=268) (R ² =0.691)	Low	63	4,796	0.035 (0.041)	0.388	AGE (p=0.005) RACE (p=0.095) ALC*DRKYR (p=0.022)
	Medium	136	4,962			
	High	69	5,305			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm unstimulated MLC response versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-16. (Continued)
Analysis of Unstimulated MLC Response (cpm)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=193) (R ² =0.743)	≤18.6	3,561 (22)	4,717 (49)	5,056 (23)	0.082 (0.079)	0.881 ^c 0.300 ^d	
	>18.6	4,231 (25)	4,327 (46)	4,824 (28)	0.065 (0.081)	0.424 ^d	
f) Maximal (n=269) (R ² =0.648)	≤18.6	3,497 (37)	4,284 (70)	4,342 (31)	0.066 (0.061)	0.621 ^c 0.283 ^d	
	>18.6	2,981 (24)	4,038 (67)	4,690 (40)	0.109 (0.061)	0.075 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=192) (R ² =0.797)	≤18.6	**** (22)	**** (49)	**** (23)	****	****	CURR*TIME*DRKYR (p=0.004)
	>18.6	**** (25)	**** (45)	**** (28)	****	****	AGE (p=0.014)
h) Maximal (n=268) (R ² =0.709)	≤18.6	4,637** (37)	5,633** (70)	5,044** (31)	0.014 (0.061)**	0.391*** 0.823*** ^d	CURR*TIME*PACKYR (p=0.016)
	>18.6	4,165** (24)	5,143** (66)	5,638** (40)	0.087 (0.060)**	0.153*** ^d	AGE (p=0.009) RACE (p=0.044) ALC*DRKYR (p=0.005)

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm unstimulated MLC response versus log₂ dioxin.

^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).

**Log₂ (current dioxin)-by-time-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

****Log₂ (current dioxin)-by-time-by-covariate interaction (p<0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-16. (Continued)
Analysis of Unstimulated MLC Response (cpm)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	294	3,619	All Categories		0.070
Unknown	124	3,691	Unknown vs. Background	72 --	0.820
Low	72	4,065	Low vs. Background	446 --	0.269
High	71	4,773	High vs. Background	1,154 --	0.011
Total	561		(R ² =0.443)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	293	3,862	All Categories		0.160	AGE*DRKYR (p=0.031) RACE*PACKYR (p=0.049)
Unknown	124	4,160	Unknown vs. Background	298 --	0.376	
Low	71	4,420	Low vs. Background	558 --	0.179	
High	71	4,797	High vs. Background	935 --	0.038	
Total	559		(R ² =0.503)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fp-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

In the adjusted analysis of unstimulated MLC under the minimal assumption, there was a significant interaction of current dioxin, time since tour, and lifetime alcohol history (Table 16-16 [g]: $p=0.004$). To investigate the interaction, adjusted analyses were performed for Ranch Hands with lifetime alcohol values of at most 40 drink-years and over 40 drink-years. For both subgroups of Ranch Hands, the interaction of current dioxin and time was nonsignificant (Appendix Table O-1: $p=0.279$ and $p=0.159$, respectively). However, for Ranch Hands with more than 40 drink-years, there was a marginally significant positive association ($p=0.059$) between unstimulated MLC response and current dioxin for time less than or equal to 18.6 years.

Under the maximal assumption, the adjusted analysis of unstimulated MLC contained a significant interaction for current dioxin, time, and lifetime cigarette smoking history (Table 16-16 [h]: $p=0.016$). To examine the interaction, adjusted analyses were performed for Ranch Hands with lifetime smoking values of 0 pack-years, 10 pack-years or less, and over 10 pack-years. For the nonsmokers, the interaction between current dioxin and time was significant (Appendix Table O-1: $p=0.041$). For this subgroup of Ranch Hands, there was a nonsignificant negative association between unstimulated MLC response and current dioxin for time of 18.6 years or less ($p=0.750$) but a significant positive association for time over 18.6 years ($p=0.008$). The interactions for the other two lifetime cigarette smoking history strata were nonsignificant ($p=0.781$ and $p=0.312$, respectively). A followup model without the interaction of current dioxin, time, and lifetime cigarette smoking history displayed a nonsignificant current dioxin-by-time interaction (Table 16-16 [h]: $p=0.391$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis of unstimulated MLC exhibited a marginally significant overall contrast among the Ranch Hand and Comparison current dioxin categories (Table 16-16 [i]: $p=0.070$). The unadjusted means of unstimulated MLC for the background, unknown, low, and high categories were 3,619 cpm, 3,691 cpm, 4,065 cpm, and 4,773 cpm. The contrast for Ranch Hands in the high current dioxin category versus Comparisons in the background current dioxin category was significant ($p=0.011$).

The adjusted analysis of unstimulated MLC displayed a nonsignificant overall contrast for the four current dioxin categories (Table 16-16 [j]: $p=0.160$). The contrast of the high versus background current dioxin categories remained significant ($p=0.038$).

MLC Net Response

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of MLC net response, the association with initial dioxin was nonsignificant under both the minimal and maximal assumptions (Table 16-17 [a] and [b]: $p=0.977$ and $p=0.922$).

Under both the minimal and maximal assumptions, the adjusted analysis of the association between MLC net response and initial dioxin was also not significant (Table 16-17 [c] and [d]: $p=0.649$ and $p=0.779$, respectively).

TABLE 16-17.
Analysis of MLC Net Response (cpm)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean	Slope (Std. Error) ^a	p-Value
a) Minimal (n=193) (R ² =0.714)	Low	45	84,357	96 (3,382)	0.977
	Medium	97	98,647		
	High	51	90,587		
b) Maximal (n=269) (R ² =0.665)	Low	63	92,445	215 (2,193)	0.922
	Medium	137	91,511		
	High	69	90,007		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
c) Minimal (n=192) (R ² =0.743)	Low	45	83,597	1,549 (3,395)	0.649	DRKYR*PACKYR (p=0.022)
	Medium	96	97,312			
	High	51	92,884			
d) Maximal (n=268) (R ² =0.696)	Low	63	92,393	597 (2,124)	0.779	ALC*PACKYR (p=0.003)
	Medium	136	93,429			
	High	69	91,892			

^aSlope and standard error based on MLC net response versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-17. (Continued)
Analysis of MLC Net Response (cpm)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean/(n) Current Dioxin			Slope (Std. Error) ^a	p-Value	
		Low	Medium	High			
e) Minimal (n=193) (R ² =0.717)	≤18.6	77,912 (22)	97,893 (49)	87,102 (23)	1,066 (4,824)	0.537 ^b 0.826 ^c	
	>18.6	98,313 (25)	95,852 (46)	93,045 (28)	-3,186 (4,976)	0.524 ^c	
f) Maximal (n=269) (R ² =0.665)	≤18.6	92,142 (37)	91,847 (70)	89,781 (31)	-778 (3,329)	0.826 ^b 0.816 ^c	
	>18.6	79,549 (24)	99,662 (67)	86,117 (40)	286 (3,337)	0.932 ^c	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean/(n) Current Dioxin			Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=192) (R ² =0.755)	≤18.6	82,939 (22)	98,003 (49)	85,843 (23)	-753 (4,657)	0.936 ^b 0.872 ^c	ALC*PACKYR (p=0.008)
	>18.6	92,046 (25)	97,181 (45)	97,402 (28)	-1,291 (4,873)	0.792 ^c	
h) Maximal (n=268) (R ² =0.697)	≤18.6	94,073 (37)	94,606 (70)	90,730 (31)	-1,356 (3,210)	0.487 ^b 0.673 ^c	ALC*PACKYR (p=0.003)
	>18.6	77,114 (24)	101,149 (66)	88,714 (40)	1,914 (3,270)	0.559 ^c	

^aSlope and standard error based on MLC net response versus log₂ dioxin.

^bTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

^cTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-17. (Continued)
Analysis of MLC Net Response (cpm)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.)	p-Value
Background	294	88,293	All Categories		0.582
Unknown	124	89,021	Unknown vs. Background	728 (-8,397, 9,853)	0.876
Low	72	91,936	Low vs. Background	3,643 (-7,347, 14,632)	0.516
High	71	82,307	High vs. Background	-5,986 (-17,381, 5,409)	0.304
Total	561		(R ² =0.558)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	294	95,904	All Categories		0.528	RACE (p=0.040) CSMOK (p=0.115)
Unknown	124	97,140	Unknown vs. Background	1,236 (-7,848, 10,320)	0.790	PACKYR (p=0.149)
Low	71	100,908	Low vs. Background	5,004 (-5,950, 15,958)	0.371	ALC (p=0.066)
High	71	90,642	High vs. Background	-5,263 (-16,549, 6,024)	0.361	
Total	560		(R ² =0.572)			

Note: Background (Comparisons): Current Dioxin ≤ 10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤ 10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤ 33.3 ppt.
High (Ranch Hands): Current Dioxin > 33.3 ppt.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

For both assumptions, the unadjusted and adjusted analyses of MLC net response contained nonsignificant interactions between current dioxin and time since tour (Table 16-17 [e-h]: $p > 0.400$ for all). Analyses within time strata were not significant.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the unadjusted and adjusted analysis of the MLC net response, the overall contrasts of the four current dioxin categories were not significant (Table 16-17 [i] and [j]: $p=0.582$ and $p=0.528$, respectively). All other analyses between individual Ranch Hand versus Comparison dioxin categories were also nonsignificant.

NKCA 50/1 Net Response

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of NKCA 50/1 net response, the association with initial dioxin was nonsignificant under both the minimal and maximal assumptions (Table 16-18 [a] and [b]: $p=0.946$ and $p=0.629$).

Under both the minimal and maximal assumptions, the adjusted analyses were nonsignificant for an association between NKCA 50/1 net response and initial dioxin (Table 16-18 [c] and [d]: $p=0.970$ and $p=0.526$, respectively).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of NKCA 50/1 net response, the interactions of current dioxin and time since tour were nonsignificant under both assumptions (Table 16-18 [e] and [f]: $p=0.480$ and $p=0.277$, respectively).

In the adjusted analysis of NKCA 50/1 net response, the interaction of current dioxin and time was nonsignificant (Table 16-18 [g]: $p=0.253$) under the minimal assumption.

Under the maximal assumption, the current dioxin-by-time interaction was marginally significant in the adjusted analysis of NKCA 50/1 net response (Table 16-18 [h]: $p=0.060$). For this model, current alcohol use and an interaction between current cigarette smoking and lifetime cigarette smoking history were retained in the adjusted model. For Ranch Hands with time of 18.6 years or less, there was a nonsignificant positive association with current dioxin ($p=0.394$). For time over 18.6 years, there was a marginally significant negative association between NKCA 50/1 net response and current dioxin ($p=0.067$). For the latter time stratum, the NKCA 50/1 net response adjusted means for low, medium, and high current dioxin were 437.7, 411.4, and 387.5 cpm.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the unadjusted and adjusted analysis of the NKCA 50/1 net response, the overall contrasts of the four current dioxin categories were not significant (Table 16-18 [i] and [j]: $p=0.266$ and $p=0.299$, respectively).

TABLE 16-18.
Analysis of NKCA 50/1 Net Response (cpm)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean	Slope (Std. Error) ^a	p-Value
a) Minimal (n=191) (R ² =0.342)	Low	44	428.0	1.0 (15.4)	0.946
	Medium	95	374.9		
	High	52	428.6		
b) Maximal (n=268) (R ² =0.380)	Low	64	432.0	-4.8 (9.9)	0.629
	Medium	133	396.1		
	High	71	409.4		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
c) Minimal (n=190) (R ² =0.457)	Low	44	495.0	0.5 (14.2)	0.970	RACE (p=0.062) PACKYR (p=0.064) ALC (p<0.001)
	Medium	94	465.0			
	High	52	502.8			
d) Maximal (n=267) (R ² =0.445)	Low	64	430.1	-6.0 (9.4)	0.526	PACKYR (p=0.021) ALC (p<0.001)
	Medium	132	401.5			
	High	71	405.4			

^aSlope and standard error based on NKCA 50/1 net response versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-18. (Continued)
Analysis of NKCA 50/1 Net Response (cpm)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean/(n) Current Dioxin			Slope (Std. Error) ^a	p-Value	
		Low	Medium	High			
e) Minimal (n=191) (R ² =0.355)	≤18.6	467.7 (21)	376.0 (47)	501.4 (22)	19.3 (21.8)	0.480 ^b 0.380 ^c	
	>18.6	367.8 (25)	378.0 (47)	405.5 (29)	-1.9 (21.7)	0.932 ^c	
f) Maximal (n=268) (R ² =0.385)	≤18.6	438.9 (38)	398.6 (67)	441.7 (30)	9.6 (14.9)	0.277 ^b 0.521 ^c	
	>18.6	409.7 (24)	394.6 (67)	394.7 (42)	-13.1 (14.5)	0.368 ^c	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean/(n) Current Dioxin			Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=190) (R ² =0.467)	≤18.6	516.1 (21)	463.9 (47)	569.8 (22)	21.8 (20.1)	0.253 ^b 0.278 ^c	RACE (p=0.068) PACKYR (p=0.080) ALC (p<0.001)
	>18.6	453.5 (25)	481.8 (46)	472.1 (29)	-9.9 (20.4)	0.629 ^c	
h) Maximal (n=267) (R ² =0.465)	≤18.6	426.4 (38)	391.1 (67)	432.5 (30)	12.0 (14.1)	0.060 ^b 0.394 ^c	ALC (p<0.001) CSMOK*PACKYR (p=0.036)
	>18.6	437.7 (24)	411.4 (66)	387.5 (42)	-25.6 (13.9)	0.067 ^c	

^aSlope and standard error based on NKCA 50/1 net response versus log₂ dioxin.

^bTest of significance for current dioxin-by-time interaction (current dioxin and time continuous).

^cTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-18. (Continued)
Analysis of NKCA 50/1 Net Response (cpm)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted						
Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.) p-Value		
Background	291	416.7	All Categories			0.266
Unknown	126	423.3	Unknown vs. Background	6.6 (-35.5,48.7)		0.759
Low	71	373.6	Low vs. Background	-43.1 (-95.2,9.0)		0.106
High	72	387.9	High vs. Background	-28.8 (-82.2,24.6)		0.291
Total	560		(R ² =0.347)			
j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted						
Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.) p-Value		Covariate Remarks
Background	291	414.2	All Categories		0.299	ALC (p=0.021) CSMOK*PACKYR (p=0.003)
Unknown	126	425.2	Unknown vs. Background	11.0 (-30.5,52.6)	0.604	
Low	70	377.8	Low vs. Background	-36.4 (-87.9,15.1)	0.167	
High	72	386.3	High vs. Background	-27.9 (-80.2,24.5)	0.297	
Total	559		(R ² =0.377)			

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

NKCA 50/1 Percent Release

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of NKCA 50/1 percent release and initial dioxin, the association was not significant under both the minimal and maximal assumptions (Table 16-19 [a] and [b]: $p=0.813$ and $p=0.575$).

Under the minimal assumption, there was a significant interaction between initial dioxin and current cigarette smoking and a significant interaction between initial dioxin and lifetime alcohol history (Table 16-19 [c]: $p=0.036$ and $p=0.037$, respectively). To investigate the interactions, the four categories of current smoking (never, former, 20 cigarettes or less per day, and over 20 cigarettes per day) were examined with two categories of dichotomized lifetime alcohol history (less than or equal to 40 drink-years and greater than 40 drink-years). For Ranch Hands who never smoked, and Ranch Hands who were former smokers but had more than 40 drink-years of lifetime alcohol history, there were nonsignificant negative associations between NKCA 50/1 percent release and initial dioxin (Appendix Table O-1). For the other strata combinations of current cigarette smoking and lifetime alcohol history, there were nonsignificant positive associations. Without the two interactions in the model, the association between NKCA 50/1 percent release and initial dioxin was not significant (Table 16-19 [c]: $p=0.748$).

Under the maximal assumption, NKCA 50/1 percent release and initial dioxin were not significantly associated (Table 16-19 [d]: $p=0.714$).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

For both the minimal and maximal assumptions, the unadjusted analysis of NKCA 50/1 percent release did not contain a significant interaction between current dioxin and time since tour (Table 16-19 [e] and [f]: $p=0.735$ and $p=0.745$, respectively); thus, the slopes did not differ significantly between time strata.

For each assumption, the adjusted analysis of NKCA 50/1 percent release also indicated that the current dioxin-by-time interaction was not significant (Table 16-19 [g] and [h]: $p=0.465$ and $p=0.558$, respectively); therefore, the adjusted slopes did not differ significantly between time strata.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

In the unadjusted analysis of the NKCA 50/1 percent release, the overall contrast of the four current dioxin categories was not significant (Table 16-19 [i]: $p=0.199$). The mean NKCA 50/1 percent release for Ranch Hands in the low current dioxin category was marginally lower than that of Comparisons in the background current dioxin category ($p=0.072$, 32.4 percent versus 35.9 percent).

The adjusted analysis of NKCA 50/1 percent release contained a nonsignificant overall contrast of the four current dioxin categories (Table 16-19 [j]: $p=0.202$).

TABLE 16-19.
Analysis of NKCA 50/1 Percent Release

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean	Slope (Std. Error) ^a	p-Value
a) Minimal (n=191) (R ² <0.001)	Low	44	34.8	0.228 (0.960)	0.813
	Medium	95	32.7		
	High	52	35.6		
b) Maximal (n=268) (R ² =0.001)	Low	64	36.2	-0.391 (0.695)	0.575
	Medium	133	33.8		
	High	71	34.7		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
c) Minimal (n=190) (R ² =0.144)	Low	44	38.5**	0.293 (0.912)**	0.748**	INIT*CSMOK (p=0.036) INIT*DRKYR (p=0.037) RACE (p=0.073) ALC (p<0.001)
	Medium	94	37.8**			
	High	52	39.7**			
d) Maximal (n=267) (R ² =0.060)	Low	64	35.8	-0.247 (0.674)	0.714	CSMOK (p=0.013) ALC (p=0.001)
	Medium	132	34.2			
	High	71	34.8			

^aSlope and standard error based on NKCA 50/1 percent release versus log₂ dioxin.

**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-19. (Continued)
Analysis of NKCA 50/1 Percent Release

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean/(n) Current Dioxin			Slope (Std. Error) ^a	p-Value	
		Low	Medium	High			
e) Minimal (n=191) (R ² =0.003)	≤18.6	36.9 (21)	32.7 (47)	36.6 (22)	0.718 (1.498)	0.735 ^b 0.632 ^c	
	>18.6	34.5 (25)	31.5 (47)	35.4 (29)	0.047 (1.292)	0.971 ^c	
f) Maximal (n=268) (R ² =0.002)	≤18.6	36.8 (38)	34.3 (67)	34.2 (30)	-0.062 (1.040)	0.745 ^b 0.953 ^c	
	>18.6	35.5 (24)	33.4 (67)	34.8 (42)	-0.528 (0.987)	0.593 ^c	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean/(n) Current Dioxin			Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=190) (R ² =0.124)	≤18.6	38.9 (21)	37.6 (47)	41.6 (22)	1.491 (1.472)	0.465 ^b 0.313 ^c	AGE (p=0.104) RACE (p=0.091) PACKYR (p=0.064)
	>18.6	38.5 (25)	37.3 (46)	39.3 (29)	0.119 (1.276)	0.926 ^c	ALC (p<0.001)
h) Maximal (n=267) (R ² =0.061)	≤18.6	35.9 (38)	34.4 (67)	34.0 (30)	0.186 (1.009)	0.558 ^b 0.854 ^c	CSMOK (p=0.012) ALC (p=0.001)
	>18.6	36.1 (24)	34.0 (66)	35.1 (42)	-0.631 (0.957)	0.510 ^c	

^aSlope and standard error based on NKCA 50/1 percent release versus log₂ dioxin.

^bTest of significance for current dioxin-by-time interaction (current dioxin and time continuous).

^cTest of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-19. (Continued)
Analysis of NKCA 50/1 Percent Release

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.)	p-Value
Background	291	35.9	All Categories		0.199
Unknown	126	36.8	Unknown vs. Background	0.9 (-2.2,4.0)	0.562
Low	71	32.4	Low vs. Background	-3.6 (-7.4,0.3)	0.072
High	72	34.6	High vs. Background	-1.3 (-5.2,2.5)	0.499
Total	560		(R ² =0.008)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	291	35.2	All Categories		0.202	ALC (p=0.037) RACE*CSMOK (p=0.006)
Unknown	126	36.6	Unknown vs. Background	1.4 (-1.7,4.5)	0.364	CSMOK*PACKYR
Low	70	32.2	Low vs. Background	-3.0 (-6.8,0.9)	0.132	(p=0.023)
High	72	33.7	High vs. Background	-1.5 (-5.3,2.3)	0.447	
Total	559		(R ² =0.049)			

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

NKCI 50/1 Net Response

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of the NKCI 50/1 net response, the association with initial dioxin was nonsignificant for both the minimal and maximal assumptions (Table 16-20 [a] and [b]: $p=0.790$ for each).

Under both assumptions, the adjusted analysis of NKCI 50/1 net response were nonsignificant (Table 16-20 [c] and [d]: $p=0.551$ and $p=0.665$, respectively).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of NKCI 50/1 net response, the interaction between current dioxin and time since tour was not significant under the minimal assumption (Table 16-20 [e]: $p=0.151$).

Under the maximal assumption, the unadjusted analysis of NKCI 50/1 net response contained a marginally significant interaction between current dioxin and time (Table 16-20 [f]: $p=0.056$). For Ranch Hands with time of 18.6 years or less, there was a marginally significant positive association between NKCI 50/1 net response and current dioxin ($p=0.080$). Within this time stratum, the average NKCI 50/1 net responses were 806.1, 789.3, and 854.8 cpm for low, medium, and high current dioxin. For Ranch Hands with time over 18.6 years, there was a nonsignificant negative association ($p=0.312$).

The adjusted analysis of NKCI 50/1 net response contained a significant interaction among current dioxin, time, and race (Table 16-20 [g]: $p=0.040$) under the minimal assumption. To explore the interaction, adjusted analyses were performed for Blacks and non-Blacks separately (Appendix Table O-1). For Blacks, the interaction and time strata associations were reported and are based on sparse numbers within current dioxin and time categories. For non-Blacks, the current dioxin-by-time interaction was significant ($p=0.033$). For non-Black Ranch Hands with time of 18.6 years or less, there was a significant positive association with current dioxin ($p=0.015$) and a nonsignificant negative association with current dioxin for the other time stratum ($p=0.680$). A followup model without the interaction exhibited a marginally significant current dioxin-by-time interaction (Table 16-20 [g]: $p=0.073$). For Ranch Hands with time of 18.6 years or less, there was a significant positive association between NKCI 50/1 net response and current dioxin ($p=0.027$). For that time stratum, the average NKCI 50/1 net response for low, medium, and high current dioxin were 808.6, 798.7, and 910.7 cpm. For Ranch Hands with more than 18.6 years since their tour, there was a nonsignificant negative association ($p=0.886$).

In the adjusted analysis of NKCI 50/1 net response under the maximal assumption, there was a significant current dioxin-by-time-by-race interaction ($p=0.008$). Similar to the interaction analyses under the minimal assumption, adjusted analyses were again performed for Blacks and non-Blacks separately. For Blacks, the interaction and the time strata associations were reported and are based on sparse numbers (Appendix Table O-1). For non-Black Ranch Hands, there was a significant interaction for current dioxin and time ($p=0.017$). Non-Black Ranch Hands whose time since tour was 18.6 years or less displayed a significant positive association between NKCI 50/1 net response and current dioxin

TABLE 16-20.

Analysis of NKCI 50/1 Net Response (cpm)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean	Slope (Std. Error) ^a	p-Value
a) Minimal (n=195) (R ² =0.896)	Low	44	817.2	2.6 (9.7)	0.790
	Medium	97	806.2		
	High	54	830.9		
b) Maximal (n=270) (R ² =0.898)	Low	63	802.9	1.6 (5.8)	0.790
	Medium	134	803.7		
	High	73	801.1		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
c) Minimal (n=194) (R ² =0.903)	Low	44	812.2	5.7 (9.6)	0.551	DRKYR (p=0.112)
	Medium	96	806.4			
	High	54	838.6			
d) Maximal (n=269) (R ² =0.903)	Low	63	803.5	2.5 (5.7)	0.665	DRKYR (p=0.068)
	Medium	133	803.1			
	High	73	802.2			

^aSlope and standard error based on NKCI 50/1 net response versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-20. (Continued)
Analysis of NKCI 50/1 Net Response (cpm)

Ranch Hands - Log₂ (Current Dioxin) and Time - Unadjusted

Assumption	Time (Yrs.)	Mean/(n) Current Dioxin			Slope (Std. Error) ^a	p-Value
		Low	Medium	High		
e) Minimal (n=195) (R ² =0.899)	≤18.6	830.6 (22)	798.5 (49)	887.8 (23)	19.4 (14.1)	0.151 ^b 0.172 ^c
	>18.6	818.8 (24)	806.5 (47)	791.9 (30)	-9.0 (14.0)	0.524 ^c
f) Maximal (n=270) (R ² =0.901)	≤18.6	806.1 (38)	789.3 (70)	854.8 (31)	15.8 (8.9)	0.056 ^b 0.080 ^c
	>18.6	826.7 (23)	791.1 (65)	785.0 (43)	-9.1 (8.9)	0.312 ^c

Ranch Hands - Log₂ (Current Dioxin) and Time - Adjusted

Assumption	Time (Yrs.)	Adj. Mean/(n) Current Dioxin			Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=194) (R ² =0.916)	≤18.6	808.6** (22)	798.7** (49)	910.7** (23)	32.6 (14.5)**	0.073*** ^b 0.027*** ^c	CURR*TIME*RACE (p=0.040)
	>18.6	797.2** (24)	798.4** (46)	802.3** (30)	-2.0 (14.0)**	0.886*** ^c	AGE (p=0.102) DRKYR (p=0.028)
h) Maximal (n=269) (R ² =0.913)	≤18.6	**** (38)	**** (70)	**** (31)	****	****	CURR*TIME*RACE (p=0.008)
	>18.6	**** (23)	**** (64)	**** (43)	****	****	DRKYR (p=0.028)

^aSlope and standard error based on NKCI 50/1 net response versus log₂ dioxin.

^bTest of significance for current dioxin-by-time interaction (current dioxin and time continuous).

^cTest of significance for slope different from 0 (current dioxin continuous, time categorized).

**Log₂ (current dioxin)-by-time-by-covariate interaction (0.01 < p ≤ 0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

****Log₂ (current dioxin)-by-time-by-covariate interaction (p ≤ 0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-20. (Continued)
Analysis of NKCI 50/1 Net Response (cpm)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.)	p-Value
Background	298	808.9	All Categories		0.843
Unknown	123	802.3	Unknown vs. Background	-6.6 (-32.6,19.4)	0.620
Low	72	817.5	Low vs. Background	8.6 (-23.1,40.2)	0.596
High	74	802.6	High vs. Background	-6.3 (-38.5,25.9)	0.701
Total	567		(R ² =0.819)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	298	****	All Categories		****	DXCAT*RACE (p=0.016)
Unknown	123	****	Unknown vs. Background	****	****	DXCAT*ALC (p<0.001)
Low	71	****	Low vs. Background	****	****	RACE*PACKYR (p<0.001)
High	74	****	High vs. Background	****	****	RACE*DRKYR (p=0.018)
Total	566		(R ² =0.845)			CSMOK*PACKYR (p=0.020)

**** Categorized current dioxin-by-covariate interaction (p≤0.01); adjusted mean, confidence interval, and p-value not presented.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

($p=0.020$). For the other time stratum, the association between NKCI 50/1 net response and current dioxin was negative and nonsignificant ($p=0.271$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis of NKCI 50/1 net response indicated that the overall contrast of the current dioxin categories for Ranch Hands and Comparisons was nonsignificant (Table 16-20 [i]: $p=0.843$).

The adjusted analysis of NKCI 50/1 net response contained a significant interaction between categorized current dioxin and race and an interaction between categorized current dioxin and current alcohol use (Table 16-20 [j]: $p=0.016$ and $p<0.001$, respectively). Because of sparse data on Blacks, the interactions were explored only for non-Blacks having one drink or less per day and non-Blacks having more than one drink per day. For non-Blacks who had one drink or less per day, the overall contrast of the current dioxin categories was nonsignificant (Appendix Table O-1: $p=0.387$). For non-Blacks who had more than one drink per day, the overall contrast of current dioxin categories was significant ($p=0.010$) and Ranch Hands with low current dioxin had a significantly higher NKCI 50/1 average net response than did the Comparisons with background current dioxin ($p=0.002$).

NKCI 50/1 Percent Release

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

For the unadjusted analyses of NKCI 50/1 percent release, the association with initial dioxin was not significant under both assumptions (Table 16-21 [a] and [b]: $p=0.894$ and $p=0.758$, respectively).

The adjusted analysis of NKCI 50/1 percent release exhibited nonsignificant associations with initial dioxin under both assumptions (Table 16-21 [c] and [d]: $p=0.345$ and $p=0.421$, respectively).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

The unadjusted analysis of NKCI 50/1 percent release exhibited a nonsignificant interaction between current dioxin and time since tour under the minimal assumption (Table 16-21 [e]: $p=0.176$).

Under the maximal assumption, the unadjusted analysis contained a marginally significant interaction between current dioxin and time (Table 16-21 [f]: $p=0.063$). For Ranch Hands with time of 18.6 years or less, there was a marginally significant positive association with current dioxin ($p=0.077$) and a nonsignificant negative association ($p=0.355$) with current dioxin for Ranch Hands with earlier tours of duty. For time of 18.6 years or less, the average NKCI 50/1 percent releases for low, medium, and high current dioxin were 65.8, 65.0, and 69.7 percent.

In the adjusted analysis of NKCI 50/1 percent release, there was a significant interaction of current dioxin, time, and race (Table 16-21 [g]: $p=0.033$) under the minimal assumption. To explore this interaction, separate analyses were performed for Blacks and

TABLE 16-21.
Analysis of NKCI 50/1 Percent Release

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean	Slope (Std. Error) ^a	p-Value
a) Minimal (n=195) (R ² =0.693)	Low	44	66.3	0.1 (0.8)	0.894
	Medium	97	64.9		
	High	54	66.9		
b) Maximal (n=270) (R ² =0.705)	Low	63	65.6	0.1 (0.5)	0.758
	Medium	134	66.0		
	High	73	65.6		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean	Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
c) Minimal (n=194) (R ² =0.722)	Low	44	65.2	0.8 (0.8)	0.345	AGE (p=0.109) DRKYR (p=0.065)
	Medium	96	65.1			
	High	54	68.1			
d) Maximal (n=269) (R ² =0.728)	Low	63	65.4	0.4 (0.5)	0.421	AGE (p=0.107) PACKYR (p=0.144) DRKYR (p=0.030)
	Medium	133	66.0			
	High	73	66.0			

^aSlope and standard error based on NKCI 50/1 percent release versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-21. (Continued)
Analysis of NKCI 50/1 Percent Release

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean/(n) Current Dioxin			Slope (Std. Error) ^a	p-Value	
		Low	Medium	High			
e) Minimal (n=195) (R ² =0.703)	≤18.6	67.7 (22)	64.5 (49)	71.4 (23)	1.4 (1.1)	0.176 ^b 0.206 ^c	
	>18.6	65.9 (24)	64.8 (47)	63.7 (30)	-0.7 (1.1)	0.531 ^c	
f) Maximal (n=270) (R ² =0.714)	≤18.6	65.8 (38)	65.0 (70)	69.7 (31)	1.3 (0.7)	0.063 ^b 0.077 ^c	
	>18.6	67.9 (23)	64.7 (65)	64.3 (43)	-0.7 (0.7)	0.355 ^c	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean/(n) Current Dioxin			Adj. Slope (Std. Error) ^a	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=194) (R ² =0.758)	≤18.6	65.6** (22)	64.3** (49)	73.2** (23)	2.6 (1.1)**	0.078** ^b 0.025** ^c	CURR*TIME*RACE (p=0.033) AGE (p=0.052)
	>18.6	63.9** (24)	63.9** (46)	64.4** (30)	-0.1 (1.1)**	0.942** ^c	DRKYR (p=0.018)
h) Maximal (n=269) (R ² =0.756)	≤18.6	**** (38)	**** (70)	**** (31)	****	****	CURR*TIME*RACE (p=0.009) AGE (p=0.080)
	>18.6	**** (23)	**** (64)	**** (43)	****	****	PACKYR (p=0.104) DRKYR (p=0.009)

^aSlope and standard error based on NKCI 50/1 percent release versus log₂ dioxin.

^bTest of significance for current dioxin-by-time interaction (current dioxin and time continuous).

^cTest of significance for slope different from 0 (current dioxin continuous, time categorized).

**Log₂ (current dioxin)-by-time-by-covariate interaction (0.01 < p ≤ 0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of interaction.

****Log₂ (current dioxin)-by-time-by-covariate interaction (p ≤ 0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-21. (Continued)
Analysis of NKCI 50/1 Ratio Percent Release

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean	Contrast	Difference of Means (95% C.I.)	p-Value
Background	298	66.6	All Categories		0.821
Unknown	123	65.9	Unknown vs. Background	-0.7 (-2.8,1.3)	0.488
Low	72	66.8	Low vs. Background	0.2 (-2.3,2.7)	0.867
High	74	65.7	High vs. Background	-0.9 (-3.4,1.7)	0.500
Total	567		(R ² =0.499)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean	Contrast	Difference of Adj. Means (95% C.I.)	p-Value	Covariate Remarks
Background	298	61.9**	All Categories		0.845**	DXCAT*RACE (p=0.013)
Unknown	123	61.5**	Unknown vs. Background	-0.4 (-2.4,1.6)**	0.679**	DXCAT*ALC (p=0.021)
Low	71	62.3**	Low vs. Background	0.4 (-2.1,2.8)**	0.758**	DXCAT*DRKYR (p=0.027)
High	74	61.1**	High vs. Background	-0.9 (-3.3,1.6)**	0.488**	RACE*PACKYR (p<0.001)
Total	566		(R ² =0.575)			CSMOK*PACKYR (p=0.023)

**Categorized current dioxin-by-covariate interaction (0.01<p≤0.05); adjusted mean, confidence interval, and p-value derived from a model fitted after deletion of this interaction.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

non-Blacks (Appendix Table O-1). For Blacks, the interaction and time strata associations were reported and were based on sparse numbers. For the non-Blacks, the interaction of current dioxin and time was significant ($p=0.034$). For non-Blacks with time of 18.6 years or less, there was a significant positive association between NKCI 50/1 percent release and current dioxin ($p=0.013$) and a nonsignificant negative association for time over 18.6 years ($p=0.733$). A followup adjusted model without the current dioxin-by-time-by-race interaction displayed a marginally significant interaction between current dioxin and time (Table 16-21 [g]: $p=0.078$). For time of 18.6 years or less, there was a significant positive association ($p=0.025$) with current dioxin and a nonsignificant negative association ($p=0.942$) for time over 18.6 years. For the former time stratum, the average NKCI 50/1 percent releases for low, medium, and high current dioxin were 65.6, 64.3, and 73.2 percent.

Under the maximal assumption, the adjusted analysis also contained a significant interaction for current dioxin, time, and race (Table 16-21 [h]: $p=0.009$). To explore this interaction, separate analyses were again performed for Blacks and non-Blacks (Appendix Table O-1). For Blacks, the interaction and time strata associations were reported but are based on sparse numbers. For the non-Blacks, the interaction of current dioxin and time was significant ($p=0.010$). For non-Blacks with time 18.6 years or less, there was a significant positive association between NKCI 50/1 percent release and current dioxin ($p=0.007$) and a nonsignificant negative association for time over 18.6 years ($p=0.396$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis of NKCI 50/1 percent release indicated that current dioxin categories for Ranch Hands and Comparisons were not significantly different (Table 16-21 [i]: $p=0.821$).

The adjusted analysis of NKCI 50/1 percent release contained three significant interactions between a covariate and the current dioxin categories. The covariates involved in the interactions were race, current alcohol use, and lifetime alcohol history (Table 16-21 [j]: $p=0.013$, $p=0.021$, and $p=0.027$, respectively). To investigate these interactions, current alcohol use was dichotomized into one drink or less per day and over one drink per day and lifetime alcohol history was dichotomized as 40 drink-years or less and over 40 drink-years. Because of sparse numbers for Blacks, the interactions were explored only for non-Blacks.

Regardless of their lifetime alcohol history stratum, non-Blacks who had one drink or less per day displayed nonsignificant overall contrasts for NKCI 50/1 percent release (Appendix Table O-1). For non-Blacks who had more than one drink per day and had lifetime alcohol history of 40 drink-years or less, the overall contrast of current dioxin categories was nonsignificant but the low versus background contrast was marginally significant ($p=0.067$). For that contrast, Ranch Hands exceeded the Comparisons on the average NKCI 50/1 percent release. For non-Blacks who had more than one drink per day and also had a lifetime alcohol history over 40 drink-years, the overall contrast of current dioxin categories was nonsignificant. The low versus background contrast was significant ($p=0.033$) with the Ranch Hands having the higher means for NKCI 50/1 percent release.

A followup adjusted model without the three interactions was also used to examine the NKCI 50/1 ratio percent release values among categories of Ranch Hands and Comparisons.

The overall contrast of the four current dioxin categories was nonsignificant (Table 16-21 [j]: $p=0.845$). Individual contrasts were also nonsignificant.

Laboratory Examination Variables: Quantitative Studies—Quantitative Immunoglobulins

IgA

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

The unadjusted analysis under the minimal assumption exhibited a nonsignificant association between IgA and initial dioxin (Table 16-22 [a]: $p=0.109$). Under the maximal assumption, there was a significant positive association (Table 16-22 [b]: $p=0.009$). The unadjusted means for IgA were 195.7, 213.1, and 213.7 mg/dl, for the low, medium, and high initial dioxin categories.

In the adjusted analysis under the minimal assumption, there was a significant positive association between IgA and initial dioxin (Table 16-22 [c]: $p=0.019$). Age and race were covariates retained in the model. The adjusted IgA means for low, medium, and high initial dioxin were 219.0, 235.8, and 245.7 mg/dl.

Under the maximal assumption, the adjusted analysis also displayed a significant positive association (Table 16-22 [d]: $p=0.003$) with the adjusted IgA means for low, medium, and high initial dioxin at 213.5, 229.7, and 234.7 mg/dl.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of IgA under the minimal assumption, the interaction of current dioxin and time since tour was not significant (Table 16-22 [e]: $p=0.613$); thus, the slopes describing the relationship between IgA and current dioxin did not differ significantly between time strata.

Under the maximal assumption, the unadjusted analysis also contained a nonsignificant interaction between current dioxin and time (Table 16-22 [f]: $p=0.594$). However, the slope for time greater than 18.6 years was positive and was of borderline significance ($p=0.056$).

Within that time strata, the mean levels of IgA were 190.4, 225.1, and 220.5 mg/dl for low, medium, and high current dioxin.

In the adjusted analysis under the minimal assumption, there was a significant interaction among current dioxin, time, and current cigarette smoking (Table 16-22 [g]: $p=0.017$). The interaction was investigated separately for Ranch Hands who never smoked, formerly smoked, smoked 20 cigarettes or less per day, and smoked over 20 cigarettes per day. For each of these smoking strata, the current dioxin-by-time interactions were not significant. For the first two smoking strata, there were nonsignificant positive associations for both times. For the other two smoking strata, there were nonsignificant positive associations between IgA and current dioxin for time at most 18.6 years and nonsignificant negative associations for time over 18.6 years. Without the above interaction in the model,

TABLE 16-22.
Analysis of IgA (mg/dl)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=504) (R ² =0.005)	Low	127	203.7	0.027 (0.017)	0.109
	Medium	252	215.4		
	High	125	218.9		
b) Maximal (n=720) (R ² =0.009)	Low	180	195.7	0.031 (0.012)	0.009
	Medium	363	213.1		
	High	177	213.7		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=504) (R ² =0.028)	Low	127	219.0	0.040 (0.017)	0.019	AGE (p=0.010) RACE (p=0.011)
	Medium	252	235.8			
	High	125	245.7			
d) Maximal (n=715) (R ² =0.040)	Low	179	213.5	0.035 (0.012)	0.003	RACE (p=0.007) ALC (p=0.071) AGE*PACKYR (p=0.015)
	Medium	360	229.7			
	High	176	234.7			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm IgA versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-22. (Continued)

Analysis of IgA (mg/dl)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=504) (R ² =0.011)	≤18.6	211.4 (71)	197.2 (127)	213.2 (52)	0.026 (0.027)	0.613 ^c 0.339 ^d	
	>18.6	219.2 (54)	227.0 (127)	216.7 (73)	0.008 (0.022)	0.725 ^d	
f) Maximal (n=720) (R ² =0.012)	≤18.6	202.9 (104)	199.1 (189)	210.7 (80)	0.018 (0.018)	0.594 ^c 0.317 ^d	
	>18.6	190.4 (77)	225.1 (171)	220.5 (99)	0.031 (0.016)	0.056 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=504) (R ² =0.045)	≤18.6	226.1** (71)	216.1** (127)	240.8** (52)	0.046 (0.027)**	0.480*** ^c 0.096*** ^d	CURR*TIME*CSMOK (p=0.017)
	>18.6	232.5** (54)	244.3** (127)	237.5** (73)	0.021 (0.023)**	0.352*** ^d	AGE (p=0.021) RACE (p=0.016)
h) Maximal (n=715) (R ² =0.050)	≤18.6	219.5** (104)	216.3** (188)	233.5** (79)	0.027 (0.018)**	0.688*** ^c 0.147*** ^d	CURR*TIME*CSMOK (p=0.043)
	>18.6	205.4** (76)	242.3** (169)	240.2** (99)	0.036 (0.016)**	0.027*** ^d	RACE (p=0.010) ALC (p=0.071) AGE*PACKYR (p=0.030)

^aTransformed from natural logarithm scale.^bSlope and standard error based on natural logarithm IgA versus log₂ dioxin.^cTest of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-22. (Continued)

Analysis of IgA (mg/dl)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	759	212.1	All Categories		0.013
Unknown	338	195.1	Unknown vs. Background	-17.0 --	0.003
Low	192	210.0	Low vs. Background	-2.1 --	0.769
High	179	216.1	High vs. Background	4.0 --	0.604
Total	1,468		(R ² =0.007)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	759	219.4	All Categories		0.007	AGE (p=0.001) RACE (p=0.097)
Unknown	336	202.1	Unknown vs. Background	-17.3 --	0.003	CSMOK (p=0.066)
Low	190	219.3	Low vs. Background	-0.1 --	0.982	ALC (p=0.137)
High	178	227.8	High vs. Background	8.4 --	0.292	
Total	1,463		(R ² =0.021)			

^aTransformed from natural logarithm scale.^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.^fP-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

the interaction of current dioxin and time was not significant (Table 16-22 [g]: $p=0.480$). For time less than or equal to 18.6 years, there was a positive association that was marginally significant ($p=0.096$).

In the adjusted analysis under the maximal assumption, there was also a significant interaction for current dioxin, time, and current cigarette smoking (Table 16-22 [h]: $p=0.043$). Investigation of the interaction was again undertaken separately for the current cigarette smoking strata. For former smokers, the current dioxin-by-time interaction was nonsignificant. However, for time greater than 18.6 years, there was a significant positive association between IgA and current dioxin (Appendix Table O-1: $p=0.001$) and a nonsignificant positive association for time 18.6 years or less ($p=0.387$). The other smoking strata displayed nonsignificant current dioxin-by-time interactions. An adjusted model without the cited interaction term contained a nonsignificant interaction between current dioxin and time (Table 16-22 [h]: $p=0.688$). For time over 18.6 years, there was a positive association between IgA and current dioxin that was significant ($p=0.027$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis of IgA indicated that the overall contrast of Ranch Hands and Comparisons was significant (Table 16-22 [i]: $p=0.013$). The IgA means for the background, unknown, low, and high current dioxin categories were 212.1, 195.1, 210.0, and 216.1 mg/dl. The contrast of Ranch Hands in the unknown current dioxin category versus Comparisons in the background current dioxin category was significant ($p=0.003$).

The adjusted analysis of IgA also exhibited a significant overall current dioxin category contrast (Table 16-22 [j]: $p=0.007$) and a significant contrast for Ranch Hands in the unknown current dioxin category versus the Comparisons in the background current dioxin category ($p=0.003$).

IgG

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

Under the minimal and maximal assumptions, the unadjusted analysis was not significant for an association between IgG and initial dioxin (Table 16-23 [a] and [b]: $p=0.720$ and $p=0.195$, respectively).

Under the minimal cohort, the adjusted analysis contained a significant interaction between initial dioxin and lifetime alcohol history (Table 16-23 [c]: $p=0.037$). Exploring the interaction by stratification (0 drink-years, over 0 drink-years to 40 drink-years, and above 40 drink-years), the association between IgG and initial dioxin was positive but not significant for each stratum (Appendix Table O-1). Without the interaction of initial dioxin and lifetime alcohol history in the model, the association between IgG and initial dioxin was not significant (Table 16-23 [c]: $p=0.502$).

Under the maximal assumption, the adjusted analysis contained a nonsignificant association between IgG and initial dioxin (Table 16-23 [d]: $p=0.156$).

TABLE 16-23.
Analysis of IgG (mg/dl)

Ranch Hands - Log ₂ (Initial) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=504) (R ² <0.001)	Low	127	1,018.0	0.003 (0.009)	0.720
	Medium	252	1,013.3		
	High	125	1,034.2		
b) Maximal (n=720) (R ² =0.002)	Low	180	990.2	0.008 (0.006)	0.195
	Medium	363	1,020.2		
	High	177	1,013.0		

Ranch Hands - Log ₂ (Initial) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=498) (R ² =0.147)	Low	127	1,096.0**	0.006 (0.009)**	0.502**	INIT*DRKYR (p=0.037)
	Medium	248	1,118.2**			RACE (p<0.001)
	High	123	1,132.2**			AGE*ALC (p=0.029) CSMOK*PACKYR (p=0.012) PACKYR*DRKYR (p<0.001) ALC*DRKYR (p<0.001)
d) Maximal (n=711) (R ² =0.143)	Low	178	1,103.5	0.008 (0.006)	0.156	RACE (p<0.001)
	Medium	360	1,128.0			AGE*ALC (p=0.019)
	High	173	1,117.7			CSMOK*PACKYR (p=0.007) CSMOK*DRKYR (p=0.009) PACKYR*DRKYR (p<0.001) ALC*DRKYR (p<0.001)

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm IgG versus log₂ dioxin.

**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-23. (Continued)

Analysis of IgG (mg/dl)

Ranch Hands - Log ₂ (Current Dioxin) and Time - Unadjusted							
Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
		Low	Medium	High			
e) Minimal (n=504) (R ² <0.001)	≤18.6	1,041.8 (71)	1,005.5 (127)	1,037.0 (52)	0.007 (0.014)	0.815 ^c 0.612 ^d	
	>18.6	994.4 (54)	1,023.4 (127)	1,023.0 (73)	0.003 (0.012)	0.810 ^d	
f) Maximal (n=720) (R ² =0.004)	≤18.6	997.4 (104)	1,021.0 (189)	1,028.7 (80)	0.009 (0.009)	0.938 ^c 0.311 ^d	
	>18.6	962.6 (77)	1,020.3 (171)	1,013.7 (99)	0.010 (0.008)	0.212 ^d	
Ranch Hands - Log ₂ (Current Dioxin) and Time - Adjusted							
Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=498) (R ² =0.141)	≤18.6	1,116.9 (71)	1,105.7 (126)	1,132.6 (51)	0.014 (0.013)	0.429 ^c 0.290 ^d	RACE (p<0.001) AGE*ALC (p=0.016) CSMOK*PACKYR
	>18.6	1,084.1 (54)	1,125.9 (124)	1,115.9 (72)	0.0007 (0.012)	0.952 ^d	(p=0.018) PACKYR*DRKYR (p<0.001) ALC*DRKYR (p=0.002)
h) Maximal (n=711) (R ² =0.144)	≤18.6	1,104.2 (103)	1,122.6 (188)	1,144.4 (78)	0.011 (0.009)	0.811 ^c 0.208 ^d	RACE (p<0.001) AGE*ALC (p=0.019) CSMOK*PACKYR
	>18.6	1,085.9 (76)	1,133.4 (169)	1,120.0 (97)	0.009 (0.008)	0.301 ^d	(p=0.007) CSMOK*DRKYR (p=0.010) PACKYR*DRKYR (p<0.001) ALC*DRKYR (p<0.001)

^aTransformed from natural logarithm scale.^bSlope and standard error based on natural logarithm IgG versus log₂ dioxin.^cTest of significance for current dioxin-by-time interaction (current dioxin and time continuous).^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-23. (Continued)

Analysis of IgG (mg/dl)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	759	1,024.2	All Categories		0.058
Unknown	338	986.5	Unknown vs. Background	-37.7 --	0.008
Low	192	1,021.3	Low vs. Background	-2.9 --	0.874
High	179	1,020.4	High vs. Background	-3.8 --	0.837
Total	1,468		(R ² =0.005)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	757	1,120.3	All Categories		0.132	RACE (p<0.001) DRKYR (p=0.103)
Unknown	335	1,087.1	Unknown vs. Background	-33.2 --	0.028	CSMOK*PACKYR (p=0.002)
Low	190	1,122.4	Low vs. Background	2.1 --	0.915	
High	175	1,122.1	High vs. Background	1.8 --	0.927	
Total	1,457		(R ² =0.082)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fp-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of IgG under the minimal and maximal assumptions, the interaction of current dioxin and time since tour was not significant (Table 16-23 [e] and [f]: $p=0.815$ and $p=0.938$, respectively). The nonsignificant interactions indicated that the slopes did not differ significantly between time strata.

The nonsignificant results of the unadjusted analyses remained nonsignificant in the adjusted analyses (Table 16-23 [g] and [h]: $p=0.429$ and $p=0.811$, respectively) under the minimal and maximal assumptions.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis of IgG displayed a marginally significant overall contrast of Ranch Hand and Comparison current dioxin categories (Table 16-23 [i]: $p=0.058$). The IgG means for the background, unknown, low, and high current dioxin categories were 1,024.2 mg/dl, 986.5 mg/dl, 1,021.3 mg/dl, and 1,020.4 mg/dl. The contrast of Ranch Hands in the unknown current dioxin category versus Comparisons in the background current dioxin category was significant ($p=0.008$).

The adjusted analysis of IgG exhibited a nonsignificant overall category contrast (Table 16-23 [j]: $p=0.132$). However, a significant contrast for Ranch Hands in the unknown current dioxin category versus the Comparisons in the background current dioxin category ($p=0.028$) was found with the Comparisons having the higher adjusted IgG mean.

IgM

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

Under both the minimal and maximal assumptions, the unadjusted analysis of IgM displayed a nonsignificant association with initial dioxin (Table 16-24 [a] and [b]: $p=0.425$ and $p=0.471$, respectively).

Under the minimal assumption, the adjusted analysis contained a significant interaction between initial dioxin and current alcohol use (Table 16-24 [c]: $p=0.029$). Exploring the interaction within drinking stratum, there was a nonsignificant positive association (Appendix Table O-1: $p=0.183$) for Ranch Hands who had zero to one drink per day. For Ranch Hands who had more than one drink per day to four drinks per day, a significant negative association was found between IgM and initial dioxin ($p=0.024$). For Ranch Hands with more than four drinks per day, a nonsignificant negative association was found ($p=0.210$). Without the interaction of initial dioxin and current alcohol use in the model, the adjusted model exhibited a nonsignificant association between IgM and initial dioxin (Table 16-24 [c]: $p=0.902$).

The adjusted analysis under the maximal assumption displayed a nonsignificant association between IgM and initial dioxin (Table 16-24 [d]: $p=0.268$).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis under both minimal and maximal assumptions, the interaction of current dioxin and time since tour was not significant (Table 16-24 [e] and [f]: $p=0.725$ and $p=0.174$, respectively). Thus, the association between IgM and current dioxin (i.e., the

TABLE 16-24.
Analysis of IgM (mg/dl)

Ranch Hands - Log ₂ (Initial Dioxin) - Unadjusted					
Assumption	Initial Dioxin	n	Mean ^a	Slope (Std. Error) ^b	p-Value
a) Minimal (n=504) (R ² =0.001)	Low	127	113.7	0.014 (0.017)	0.425
	Medium	252	106.8		
	High	125	115.2		
b) Maximal (n=720) (R ² <0.001)	Low	180	117.3	-0.009 (0.013)	0.471
	Medium	363	109.6		
	High	177	115.4		

Ranch Hands - Log ₂ (Initial Dioxin) - Adjusted						
Assumption	Initial Dioxin	n	Adj. Mean ^a	Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
c) Minimal (n=501) (R ² =0.024)	Low	127	107.5**	0.002 (0.018)**	0.902**	INIT*ALC (p=0.029) AGE (p=0.100) RACE (p=0.049)
	Medium	250	99.5**			
	High	124	104.9**			
d) Maximal (n=711) (R ² =0.026)	Low	178	108.0	-0.015 (0.013)	0.268	AGE (p=0.048) ALC (p=0.032) DRKYR*RACE (p=0.040)
	Medium	360	102.2			
	High	173	105.5			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on natural logarithm IgM versus log₂ dioxin.

**Log₂ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-24. (Continued)

Analysis of IgM (mg/dl)

Ranch Hands - Log₂ (Current Dioxin) and Time - Unadjusted

Assumption	Time (Yrs.)	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value
		Low	Medium	High		
e) Minimal (n=504) (R ² =0.003)	≤18.6	121.0 (71)	101.0 (127)	109.5 (52)	0.0006 (0.028)	0.725 ^c 0.983 ^d
	>18.6	112.0 (54)	111.9 (127)	115.7 (73)	0.013 (0.023)	0.567 ^d
f) Maximal (n=720) (R ² =0.004)	≤18.6	125.4 (104)	105.8 (189)	111.4 (80)	-0.033 (0.020)	0.174 ^c 0.099 ^d
	>18.6	111.6 (77)	112.3 (171)	117.9 (99)	0.004 (0.018)	0.844 ^d

Ranch Hands - Log₂ (Current Dioxin) and Time - Adjusted

Assumption	Time (Yrs.)	Adj. Mean ^a /(n) Current Dioxin			Adj. Slope (Std. Error) ^b	p-Value	Covariate Remarks
		Low	Medium	High			
g) Minimal (n=498) (R ² =0.031)	≤18.6	112.7 (71)	90.9 (126)	94.3 (51)	-0.022 (0.029)	0.519 ^c 0.456 ^d	AGE (p=0.029) RACE*DRKYR (p=0.021)
	>18.6	104.4 (54)	103.5 (124)	104.7 (72)	0.002 (0.024)	0.934 ^d	
h) Maximal (n=711) (R ² =0.032)	≤18.6	114.8 (103)	97.3 (188)	99.7 (78)	-0.045 (0.020)	0.137 ^c 0.027 ^d	AGE (p=0.021) ALC (p=0.033) RACE*DRKYR (p=0.034)
	>18.6	104.4 (76)	105.0 (169)	108.5 (97)	-0.005 (0.018)	0.776 ^d	

^aTransformed from natural logarithm scale.^bSlope and standard error based on natural logarithm IgM versus log₂ dioxin.^cTest of significance for current dioxin-by-time interaction (current dioxin and time continuous).^dTest of significance for slope different from 0 (current dioxin continuous, time categorized).Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-24. (Continued)

Analysis of IgM (mg/dl)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean ^a	Contrast	Difference of Means (95% C.I.) ^e	p-Value ^f
Background	759	110.3	All Categories		0.079
Unknown	338	113.9	Unknown vs. Background	3.6 --	0.301
Low	192	103.1	Low vs. Background	-7.2 --	0.076
High	179	115.0	High vs. Background	4.7 --	0.294
Total	1,468		(R ² =0.005)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean ^a	Contrast	Difference of Adj. Means (95% C.I.) ^e	p-Value ^f	Covariate Remarks
Background	757	103.0	All Categories		0.099	AGE (p=0.005) RACE (p=0.004)
Unknown	335	106.6	Unknown vs. Background	3.6 --	0.266	ALC (p=0.005)
Low	190	96.3	Low vs. Background	-6.7 --	0.078	DRKYR (p=0.104)
High	175	105.9	High vs. Background	2.9 --	0.477	
Total	1,457		(R ² =0.021)			

^aTransformed from natural logarithm scale.

^eDifference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

^fP-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

slopes) did not differ significantly between time strata. Under the maximal assumption, a negative association between IgM and current dioxin was marginally significant ($p=0.099$) for time of 18.6 years or less.

In the adjusted analysis under the minimal assumption, the current dioxin-by-time interaction was nonsignificant (Table 16-24 [g]: $p=0.519$). Thus, the relationships between IgM and current dioxin were not significant between time strata.

In the adjusted analysis under the maximal assumption, the interaction of current dioxin and time was also not significant (Table 16-24 [h]: $p=0.137$). For time less than or equal to 18.6 years, there was a negative association between IgM and current dioxin that was significant ($p=0.027$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis of IgM indicated that the overall contrast of current dioxin categories for Ranch Hands and Comparisons was marginally significant (Table 16-24 [i]: $p=0.079$). The IgM means for the background, unknown, low, and high current dioxin categories were 110.3, 113.9, 103.1, and 115.0 mg/dl. The contrast of Ranch Hands in the low current dioxin category versus Comparisons in the background current dioxin category was marginally significant ($p=0.076$).

Similar to the unadjusted analysis of IgM, the adjusted analysis indicated that the overall contrast of Ranch Hands and Comparisons was marginally significant (Table 16-24 [j]: $p=0.099$). The contrast of Ranch Hands in the low current dioxin category versus Comparisons in the background current dioxin category was also marginally significant ($p=0.078$).

Longitudinal Analysis

Laboratory Examination Data

CD4/CD8 Ratio

For the immunologic evaluation, longitudinal analyses were conducted to examine the change in the CD4/CD8 ratio between the 1985 and the 1987 examinations for associations with initial dioxin, current dioxin and time since tour, and categorized current dioxin. Table 16-25 presents the results of these analyses. For a specific longitudinal analysis of the CD4/CD8 ratio (e.g., minimal assumption, initial dioxin analysis), the left side of each subpanel of the table provides the means and sample sizes for participants with laboratory values at each examination. Based on the difference between 1987 and 1985 laboratory values, the right side of each subpanel presents slopes, standard errors, and associated p-values (for models using initial dioxin or models using current dioxin and time since tour), or differences of examination mean changes, 95 percent confidence intervals, and associated p-values (for models using categorized current dioxin). The reported statistics for all three examinations are presented subject to the constraint that participants were at both the 1985 and 1987 examinations.

TABLE 16-25.
Longitudinal Analysis of CD4/CD8 Ratio

Ranch Hands - Log₂ (Initial Dioxin)					
Assumption	Initial Dioxin	Mean^a/(n) Examination		Slope (Std. Error)^b	p-Value
		1985	1987		
a) Minimal (R ² <0.001)	Low	1.70 (39)	1.98 (39)	0.001 (0.017)	0.968
	Medium	1.66 (88)	2.06 (88)		
	High	1.63 (47)	1.89 (47)		
b) Maximal (R ² =0.006)	Low	1.60 (51)	1.82 (51)	0.014 (0.012)	0.234
	Medium	1.62 (121)	1.97 (121)		
	High	1.67 (64)	1.94 (64)		

^aTransformed from natural logarithm scale.

^bSlope and standard error based on difference between natural logarithm of 1987 CD4/CD8 ratio and natural logarithm of 1985 CD4/CD8 ratio versus log₂ dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.

Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

Summary statistics for 1985 are provided for reference purposes for participants who attended the Baseline, 1985, and 1987 examinations. P-values given are in reference to a contrast of 1985 and 1987 results.

TABLE 16-25. (Continued)
Longitudinal Analysis of CD4/CD8 Ratio

Ranch Hands - Log₂ (Current Dioxin) and Time								
Assumption	Time (Yrs.)	Examination	Mean ^a /(n) Current Dioxin			Slope (Std. Error) ^b	p-Value	
			Low	Medium	High			
c) Minimal (R ² =0.022)	≤18.6	1985	1.75	1.57	1.60	-0.031 (0.026)	0.062 ^c 0.240 ^d	
			(20)	(43)	(20)			
		1987	2.05	2.09	1.66			
			(20)	(43)	(20)			
	>18.6	1985	1.69	1.73	1.63	0.033 (0.022)	0.131 ^d	
			(21)	(44)	(26)			
		1987	1.87	2.10	2.02			
			(21)	(44)	(26)			
d) Maximal (R ² =0.020)	≤18.6	1985	1.70	1.59	1.65	0.003 (0.018)	0.190 ^c 0.879 ^d	
			(30)	(60)	(27)			
		1987	1.93	2.01	1.84			
			(30)	(60)	(27)			
	>18.6	1985	1.40	1.69	1.66	0.035 (0.016)	0.035 ^d	
			(22)	(59)	(38)			
		1987	1.51	1.97	2.06			
			(22)	(59)	(38)			

^aTransformed from natural logarithm scale.

^bSlope and standard error based on difference between natural logarithm of 1987 CD4/CD8 ratio and natural logarithm of 1985 CD4/CD8 ratio versus log₂ dioxin.

^cTest of significance for homogeneity of slopes (current dioxin continuous, time categorized).

^dTest of significance for slope equal to 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.

Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

Summary statistics for 1985 are provided for reference purposes for participants who attended the Baseline, 1985, and 1987 examinations. P-values given are in reference to a contrast of 1985 and 1987 results.

TABLE 16-25. (Continued)
Longitudinal Analysis of CD4/CD8 Ratio

e) Ranch Hands and Comparisons by Current Dioxin Category						
Current Dioxin Category	<u>Mean^a/(n) Examination</u>		Contrast	Difference of Examination Mean Change (95% C.I.) ^e		p-Value ^f
	1985	1987				
Background	1.62 (270)	1.93 (270)	All Categories			0.404
Unknown	1.54 (104)	1.80 (104)	Unknown vs. Background	-0.04	- -	0.637
Low	1.58 (65)	2.00 (65)	Low vs. Background	0.10	- -	0.141
High	1.66 (65)	1.96 (65)	High vs. Background	-0.001	- -	0.918
(R ² =0.006)						

^aTransformed from natural logarithm scale.

^eDifference of 1987 and 1985 examination mean changes after transformation to original scale; confidence interval on difference of 1987 and 1985 examination mean changes not given because analysis was performed on natural logarithm scale.

^fP-value is based on difference of 1987 and 1985 examination mean changes on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.

Unknown (Ranch Hands): Current Dioxin ≤10 ppt.

Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.

High (Ranch Hands): Current Dioxin >33.3 ppt.

Summary statistics for 1985 are provided for reference purposes for participants who attended the Baseline, 1985, and 1987 examinations. P-values given are in reference to a contrast of 1985 and 1987 results.

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

For both the minimal and the maximal longitudinal analyses, the association between initial dioxin and the change in the CD4/CD8 ratio of the 1987 examination value relative to the 1985 examination value was nonsignificant (Table 16-25 [a] and [b]: $p=0.968$ and $p=0.234$, respectively).

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

Under the minimal assumption, the longitudinal analysis detected a marginally significant interaction between current dioxin and time since tour (Table 16-25 [c]: $p=0.062$). For Ranch Hands with less than or equal to 18.6 years since their tour, there was a nonsignificant decreasing association between current dioxin and the change in the CD4/CD8 ratio between 1985 and 1987 ($p=0.240$). In contrast, for Ranch Hands with more than 18.6 years since tour, there was a nonsignificant increasing association between current dioxin and the change in the CD4/CD8 ratio between 1985 and 1987 ($p=0.131$).

Based on the maximal assumption, the longitudinal analysis did not detect a significant interaction between current dioxin and time since tour (Table 16-25 [d]: $p=0.190$). However, for Ranch Hands with more than 18.6 years since their tour, there was a significant increasing association between current dioxin and the change in the CD4/CD8 ratio between the 1985 and 1987 examinations ($p=0.035$). The differences of the mean CD4/CD8 ratios from 1985 to 1987 were 0.11, 0.28, and 0.40 for low, medium, and high current dioxin. Thus, for this time stratum, the increases in the mean CD4/CD8 ratio in 1987, relative to 1985, were greater for higher current dioxin levels.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the longitudinal analysis of categorized current dioxin, there was no significant difference in the change in the CD4/CD8 ratio (1987 relative to 1985) among the four current dioxin categories (Table 16-25 [e]: $p=0.404$).

DISCUSSION

Background

Immunologic competence was assessed by analysis of data from cell surface marker studies, immunoglobulin quantitation, functional stimulation assays, and skin tests for delayed hypersensitivity response on a randomized subset of the study population. The absence of a response to a series of skin test antigens is usually indicative of an impaired immune defense mechanism (anergy). Anergy can occur in elderly individuals in the setting of certain viral, bacterial, and fungal infections; or with advanced protein deficiency, underlying malignancy, or treatment with corticosteroids and other immunosuppressive agents. Skin tests for delayed cutaneous hypersensitivity (DCH) are occasionally used to test for anergy as a prognostic indicator in individuals in compromised states such as the acquired immunodeficiency syndrome or those at risk of infection following surgery.

Skin tests for DCH are subject to numerous variables including the dose and method of administration of the antigen and the techniques employed in reading and interpreting the response. Following quality control concerns over the 1985 Air Force Health Study skin test

data, stringent protocols were established to ensure consistent methods and interpretation. In the current study, a premium was placed on uniform and consistent methods of interpretation. There was a 92 percent concordance between readers and duplicate interpretations by the same reader. More than 99.6 percent of the sample population had interpretable skin tests. The 94.9 percent incidence of intact DCH is consistent with clinical experience in the general population (5).

Evaluation of the human immune system is divided into separate segments for humoral and cellular immunity and each of these is further divided into measurements of quality (e.g., cell counts and protein concentrations) and function. Circulating in the plasma phase of blood, the humoral segment consists of the immunoglobulin and complement proteins some of which are also prominent at exposed sites of the body such as mucosal surfaces. The serum immunoglobulins are secreted by plasma cells in the bone marrow and are regulated in a sequence of events modulated by macrophages and memory lymphocytes. The immunoglobulins serve as a defense against bacterial infections and the blood-borne phase of viral infections.

Quantitative analysis of IgG, IgM, and IgA give an overall view of B-cell integrity when related to the expected reference range of values. Selective deficiency of one or more of these antibody classes, whether congenital or acquired, may be associated with increased susceptibility to infections. Elevations of these immunoglobulins in a polyclonal pattern are frequently an indication of chronic infections (perhaps due to impairment of another segment of the immune response) or of a faulty regulation of B-cell responses such as occurs in cirrhosis. Selective elevation of a monoclonal segment of any immunoglobulin (detected by visual examination of serum protein electrophoresis) is a strong indicator of faulty regulation or actual autonomy of plasma cells or lymphocytes and may be an early hallmark of numerous conditions including plasmacytoma, multiple myeloma, chronic lymphocytic leukemia/lymphoma, and smoldering myeloma. Occasionally there may be a cluster of more than one small spike of immunoglobulin in the presence of other normal immunoglobulins. Invariably, this type of oligoclonal banding is associated with some alteration of the immune system (e.g., primary bone marrow involvement, inappropriate regulation, or immunosuppression as in organ transplant recipients). Thus, both quantitative and qualitative parameters of the serum immunoglobulins can give information on the integrity of B-cell responses.

Cellular immunity consists of both granulocytic and lymphocytic processes. Abnormalities of granulocytes can frequently be discerned from examination of the peripheral blood smear as part of the complete blood count. In addition, the infectious history of individuals is usually sufficient to ascertain whether granulocytic deficiency is a consideration. Chapter 13, Hematologic Assessment, discusses the effect of dioxin on the components of these cells.

The lymphocytic segment of the immune response can be broadly evaluated by skin testing against multiple fungal, bacterial, or viral agents. The response to skin tests is dependent in part on the infection exposure history of the patient, and so is probably better used in the diagnosis of specific diseases than in an overall examination of lymphocyte function, although it does have the particular merit of demonstrating the presence or absence of the response in vivo where it must be effective for the patient to remain healthy.

The *in vitro* functional stimulation tests of PHA and MLC are very valuable for showing explicitly whether there is an impairment of lymphocyte function in response to mitogenic and mixed cellular antigens. Although these assays are typically used in the diagnosis of congenital deficiencies of lymphocyte function (PHA) or for the cross-matching of organ donors and recipients (MLC), they may also demonstrate abnormalities in the cellular immune response associated with a variety of different conditions. Infection with cytomegalovirus, for example, can cause a reduction in the PHA response that reverts to normal in the convalescent phase. In addition, malnutrition, some autoimmune diseases, advanced age, physical stress or trauma, and advanced malignancy can be marked by low PHA response. The assay for natural killer cells with and without IL-2 is useful in evaluating the ability of peripheral blood lymphocytes to destroy dissimilar target cells. This destructive ability has been correlated with the potential of an individual to destroy tumor cells, invasive organisms, and other foreign antigens and presumably is an index of how well an individual's immune system would be in destroying developing tumors.

The total number of circulating lymphocytes provides information relative to the basic cellular quantity of cells that is present and available in the body for mounting an immune response. Examination of the surface marker proteins on the surfaces of these lymphocytes by flow cytometry is an excellent means of evaluating whether the regulatory interactions between T cells, B cells, and monocytes are intact. An alteration in the percentages of any of these categories can be considered presumptive evidence of an inability to recognize and destroy foreign infectious agents or tumor cells. The marker for total T cells was CD2 which is further broken down into the subpopulations of CD4 (helper cells) and CD8 (suppressor cells); CD4 and CD8 should be mutually exclusive. The ratio of CD4/CD8 describes whether the regulation is in balance. Expected values for the CD4/CD8 ratio are roughly 0.9 to 3.5. Ratios substantially below 1.0 are to be expected in patients immunosuppressed with cyclosporine and also those with active human immunodeficiency virus infection that involves primarily the CD4 positive cells. Activation of T cells results in the new synthesis of IL-2 receptor molecules on the surface of lymphocytes. This IL-2 receptor is also designated CD25, and its presence in excess is an indicator of recent stimulus to the immune system by virtually any type of antigen: infectious organism, transplanted organ, etc. The surface marker for B cells, CD20, gives an indication of the balance between cellular immunity and the ability to mount a B-cell response with production of specific antibodies. The CD14 marker is specific for monocytes that are essential for the correct transfer of stimulatory information from the (foreign) antigen processing segment to the antibody turn-on segment of a B-cell response.

Interpretation of alterations in the relative amounts of B cells, T cells and their subsets, and monocytes is based on the expectation that all aspects of the immune system must be intact to prevent infections and to guard against development of tumors with unusual surface antigens. The antibodies specific for tumors can either help to destroy them by binding complement and lysing the cells or stabilize them if those antibodies attach to the tumor surface without binding complement thereby blocking immune recognition and destruction of tumor cells. The T cells also have antigen receptors on their surfaces that similarly call into play the destructive power of the entire lymphocyte cell line in an antitumor attack. T cells stimulated by IL-2 have even greater capacity to attack and destroy foreign cells. Natural killer cells have still greater destructive capacity, but they act on a nonspecific basis and are probably simply recruited into regions of foreign antigens and tumors by the other recognition factors.

Clinical Interpretation of Statistically Significant Results

Immunoglobulins

The concentrations of IgA observed in this study increased with alcohol consumption (5) and are known to occur as an expected consequence of alcoholic cirrhosis, presumably due to diminished regulation of B-cell responses. These changes in cirrhosis lead to polyclonal gamopathy with IgA particularly being synthesized out of proportion to normal. Whether the significant associations between IgA and dioxin seen here are due to a combination of effects from alcohol, tobacco, and dioxin bears examination at other phases of the AFHS. A consistent trend would help to determine the clinical interpretation of these results. The increased IgA levels could represent a chronic inflammatory response to dioxin exposure, as do elevated erythrocyte sedimentation rates (in the general health assessment) and white blood cell and platelet counts (in the hematologic assessment). The analyses of the other immunoglobulins do not indicate the presence of any dioxin-related effects.

PHA Response

PHA (a plant lectin derived from the kidney bean) is a mitogen and as such induces proliferation or blast transformation of normal lymphocytes in cell culture. This response entails induction of new deoxyribonucleic acid (DNA) synthesis that is the basis of quantifying the PHA stimulation according to the incorporation of radioactive thymidine into high molecular weight DNA. The in vitro response to mitogens correlates well with the ability of the body to mount a delayed hypersensitivity response. Various congenital defects of cellular immunity may result in a spectrum of abnormalities in lymphocyte transformation from complete to partial lack of function resulting in increased susceptibility to infections from bacteria, fungi, and viruses. Furthermore, acquired impairment of lymphocyte transformation may be associated with the stress of surgery and anesthesia, aging, malnutrition, major burns, uremia, some malignancies, and other clinical conditions. The in vitro response to mitogens is also reduced in immunosuppression (e.g., cyclosporine) and increased in immunoenhancement (e.g., treatment with interleukins or interferon).

Because the response of lymphocytes from normal individuals to PHA can vary substantially over the concentration range of the mitogen employed, the PHA assays of this study were done at three different concentrations: PHA concentration 1, 36 µg/ml; PHA concentration 2, 12 µg/ml; PHA concentration 3, 4 µg/ml. One or more of these PHA concentrations will be expected to yield maximal response from normal lymphocytes, and the highest levels of PHA will be associated with some saturation and inhibition from the maximal response. In general, the cpm values were less for PHA concentration 1 (inhibition of response) than with PHA concentration 2 and PHA concentration 3, and values for PHA concentration 2 were generally equal to those for PHA concentration 3 (saturated response). However, none of these differences should be considered clinically different from one another. An acceptable range of variability in the lymphocyte response of a normal individual is from 65 percent to 180 percent of the mean value of a group of normal individuals.

The positive associations that were found to be statistically significant for maximum PHA net response in this study are not supportive of an impaired immune response. Variation in the assay is affected by long-term potency of the mitogen preparation and because of fluctuations in the stability and activity of the lymphocytes, depends on a patient's recent activities and minor medical conditions. Fortunately this degree of normal variability

does not pose a major problem for diagnosing alterations in the immune response because true deficiencies are associated with very low cpm values, perhaps 10,000 cpm or less.

Corroborative information regarding lymphocyte transformation is obtained from the MLC assay in which the foreign mitogen is replaced by antigens from allogeneic mixed lymphocytes inactivated by irradiation. Thus, any true deficiency in lymphocyte response observed in the PHA assay should also be present in the MLC assay. The actual data indicate a statistical difference in PHA data without corroboration from the MLC analysis.

In summary, the indices of immunologic capability analyzed in the current section provide a comprehensive reflection of in vivo and in vitro immune function in the study population. No clinically significant trends were observed relative to the current body burden of dioxin or the extrapolated initial exposure.

SUMMARY

For the immunologic assessment, Tables 16-26, 16-27, and 16-28 summarize the results from analyses based on initial dioxin, current dioxin and time since tour, and categorized current dioxin. All variables, except for the composite skin test diagnosis variable, were continuous in form. Table 16-29 summarizes the covariate interactions from the three models. Each of the interactions listed in Table 16-29 was reviewed along with the corresponding interaction displays in Appendix O for medical plausibility and consistency with the current literature. Based on this review, none of these interactions indicated dioxin-mediated immunosuppression in any subgroup of Ranch Hands.

Physical Examination Variable: Composite Skin Test Diagnosis

For the composite skin test diagnosis, the unadjusted and adjusted analysis of the relative frequency of participants with possibly abnormal tests exhibited nonsignificant associations with initial dioxin. The unadjusted and adjusted analyses of the composite skin test results were also nonsignificant for the models using current dioxin and time since tour. However, the adjusted analysis for the minimal cohort contained a significant current dioxin-by-time-by-age interaction which, when investigated, did not display significant associations with current dioxin for specified age and time since tour strata. For categorized current dioxin, the Ranch Hand and Comparison group contrasts were generally nonsignificant.

Laboratory Examination Variables: Cell Surface Marker (Phenotypic) Studies

The following cell surface marker variables were analyzed using a natural logarithm transformation: CD2 cells, CD4 cells, CD8 cells, CD20 cells, CD14 cells, CD25 cells, HLA-DR cells, and the CD4/CD8 ratio.

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

Except for the CD4/CD8 ratio, which exhibited a marginally significant positive association with initial dioxin under the maximal assumption, the unadjusted analyses of the cell surface marker variables were not significantly associated with initial dioxin (see Table 16-26). A negative association would be expected in the presence of immunodeficiency. For the adjusted analyses, the minimal cohort displayed a significant initial dioxin-by-covariate

TABLE 16-26.

**Summary of Initial Dioxin Analyses for Immunology Variables Based on
Minimal and Maximal Assumptions
(Ranch Hands Only)**

Variable	Unadjusted		Adjusted	
	Minimal	Maximal	Minimal	Maximal
Physical Examination				
Composite Skin Test				
Diagnosis (D)	ns	ns	ns	ns
Laboratory				
CD2 Cells (C)	ns	NS	****	NS
CD4 Cells (C)	NS	NS	NS	NS
CD8 Cells (C)	ns	ns	****	** (ns)
CD20 Cells (C)	NS	NS	** (NS*)	NS
CD14 Cells (C)	NS	ns	****	ns
CD25 Cells (C)	ns	NS	****	****
HLA-DR Cells (C)	ns	NS	****	** (NS)
CD4/CD8 Ratio (C)	NS	NS*	NS	NS*
TLC (C)	ns	NS	** (ns)	NS
Unstimulated PHA Response (C)	NS	NS	NS	NS
PHA Net Response - Conc. 1 (C)	NS	NS	****	** (ns)
PHA Net Response - Conc. 2 (C)	+0.016	+0.008	NS*	NS*
PHA Net Response - Conc. 3 (C)	NS	NS	NS	** (NS)
PHA Net Response - Across Day and Concentration	NS*	NS	** (NS)	--
Maximum PHA Net Response (C)	+0.005	+0.009	NS*	NS*
Unstimulated MLC Response (C)	NS	NS*	NS	NS
MLC Net Response (C)	NS	NS	NS	NS
NKCA 50/1 Net Response (C)	NS	ns	NS	ns
NKCA 50/1 Percent Release (C)	NS	ns	** (NS)	ns
NKCI 50/1 Net Response (C)	NS	NS	NS	NS
NKCI 50/1 Percent Release (C)	NS	NS	NS	NS
IgA (C)	NS	+0.009	+0.019	+0.003
IgG (C)	NS	NS	** (NS)	NS
IgM (C)	NS	ns	** (NS)	ns

C: Continuous analysis.

D: Discrete analysis.

+: Relative risk 1.00 or greater for discrete analysis.

--: Not applicable.

NS/ns: Not significant ($p > 0.10$).NS*: Marginally significant ($0.05 < p \leq 0.10$).

** (NS)/** ns: \log_2 (initial dioxin)-by-covariate interaction ($0.01 < p \leq 0.05$); not significant when interaction is deleted; refer to Appendix Table O-1 for a detailed description of this interaction.

** (NS*): \log_2 (initial dioxin)-by-covariate interaction ($0.01 \leq p \leq 0.05$); marginally significant when interaction is deleted; refer to Appendix Table O-1 for a detailed description of this interaction.

****: \log_2 (initial dioxin)-by-covariate interaction ($p \leq 0.01$); refer to Appendix Table O-1 for a detailed description of this interaction.

Note: P-value given if $p \leq 0.05$.

A capital "NS" denotes slope nonnegative for continuous analysis; a lowercase "ns" denotes relative risk less than 1.00 for discrete analysis or slope negative for continuous analysis.

TABLE 16-27.

**Summary of Current Dioxin and Time Analyses for Immunology Variables
Based on Minimal and Maximal Assumptions
(Ranch Hands Only)**

Variable	Unadjusted					
	Minimal			Maximal		
	C*T	≤18.6	>18.6	C*T	≤18.6	>18.6
Physical Examination						
Composite Skin Test Diagnosis (D)	NS	ns	NS	NS	ns	ns
Laboratory						
CD2 Cells (C)	NS	ns	ns	NS	NS	NS
CD4 Cells (C)	NS	ns	NS	NS	NS	NS
CD8 Cells (C)	ns	ns	ns	ns	ns	ns
CD20 Cells (C)	NS	NS	NS	NS	NS	NS*
CD14 Cells (C)	NS	ns	NS	NS	ns	NS
CD25 Cells (C)	NS*	- 0.028	NS	NS	ns	NS
HLA-DR Cells (C)	NS	ns	NS	NS	ns	NS
CD4/CD8 Ratio (C)	ns	NS	NS	ns	NS*	NS
TLC (C)	NS	ns	NS	NS	ns	NS
Unstimulated PHA Response (C)	ns	NS	NS	ns	NS	NS
PHA Net Response (C)	ns*	+ 0.006	NS	ns	NS	NS
Maximum PHA Net Response (C)	ns	+ 0.002	NS	NS	+ 0.049	+ 0.008
Unstimulated MLC Response (C)	ns	NS	NS	NS	NS	NS*
MLC Net Response (C)	ns	NS	ns	NS	ns	NS
NKCA 50/1 Net Response (C)	ns	NS	ns	ns	NS	ns
NKCA 50/1 Percent Release (C)	ns	NS	NS	ns	ns	ns
NKCI 50/1 Net Response (C)	ns	NS	ns	ns*	NS*	ns
NKCI 50/1 Percent Release (C)	ns	NS	ns	ns*	NS*	ns
IgA (C)	ns	NS	NS	NS	NS	NS*
IgG (C)	ns	NS	NS	NS	NS	NS
IgM (C)	NS	NS	NS	NS	ns*	NS

C: Continuous analysis.

D: Discrete analysis.

+: ≤18.6 and >18.6: Slope nonnegative for continuous analysis.

-: ≤18.6 and >18.6: Slope negative for continuous analysis.

NS/ns: Not significant (p>0.10).

NS*/ns*: Marginally significant (0.05<p≤0.10).

Notes: P-value given if p≤0.05.

C*T: Log₂ (current dioxin)-by-time interaction hypothesis test.≤18.6: Log₂ (current dioxin) hypothesis test for Ranch Hands with time since end of tour of 18.6 years or less.>18.6: Log₂ (current dioxin) hypothesis test for Ranch Hands with time since end of tour greater than 18.6 years.

A capital "NS" denotes relative risk/slope for ≤18.6 category less than relative risk/slope for >18.6 category, relative risk 1.00 or greater for discrete analysis, or slope nonnegative for continuous analysis; a lowercase "ns" denotes slope for ≤18.6 category greater than slope for >18.6 category, relative risk less than 1.00 for discrete analysis, or slope negative for continuous analysis.

TABLE 16-27. (Continued)
Summary of Current Dioxin and Time Analyses for Immunology Variables
Based on Minimal and Maximal Assumptions
(Ranch Hands Only)

Variable	Minimal			Adjusted		
	C*T	≤18.6	>18.6	C*T	≤18.6	>18.6
Physical Examination						
Composite Skin Test Diagnosis (D)	** (NS)	** (ns)	** (ns)	NS	ns	ns
Laboratory						
CD2 Cells (C)	NS	ns	ns	NS	ns	NS
CD4 Cells (C)	** (NS)	** (ns)	** (NS)	** (NS)	** (ns)	** (NS)
CD8 Cells (C)	NS	ns	ns	ns	ns	ns
CD20 Cells (C)	NS	NS	+ 0.043	NS	ns	NS*
CD14 Cells (C)	NS	ns	NS	****	****	****
CD25 Cells (C)	NS*	- 0.028	NS	NS	ns	NS
HLA-DR Cells (C)	NS	ns	NS	NS	ns	NS
CD4/CD8 Ratio (C)	ns	NS	ns	ns	NS*	NS
TLC (C)	NS*	ns	NS	NS	ns	NS
Unstimulated PHA Response (C)	NS	ns	NS	ns	NS	NS
PHA Net Response (C)	** (ns*)	** (+0.033)	** (ns)	ns	NS	NS
Maximum PHA Net Response (C)	ns	+0.013	NS	NS	NS	NS*
Unstimulated MLC Response (C)	****	****	****	** (NS)	** (NS)	** (NS)
MLC Net Response (C)	ns	ns	ns	NS	ns	NS
NKCA 50/1 Net Response (C)	ns	NS	ns	ns*	NS	ns*
NKCA 50/1 Percent Release (C)	ns	NS	NS	ns	NS	ns
NKCI 50/1 Net Response (C)	** (ns*)	** (+0.027)	** (ns)	****	****	****
NKCI 50/1 Percent Release (C)	** (ns*)	** (+0.025)	** (ns)	****	****	****
IgA (C)	** (ns)	** (NS*)	** (NS)	** (NS)	** (NS)	** (+0.027)
IgG (C)	ns	NS	NS	ns	NS	NS
IgM (C)	NS	ns	NS	NS	- 0.027	ns

C: Continuous analysis.

D: Discrete analysis.

+: ≤18.6 and >18.6: Slope nonnegative for continuous analysis.

-: ≤18.6 and >18.6: Slope negative for continuous analysis.

NS/ns: Not significant ($p > 0.10$).

NS*/ns*: Marginally significant ($0.05 < p \leq 0.10$).

** (NS)/** (ns): Log₂ (current dioxin)-by-time-by-covariate interaction ($0.01 < p \leq 0.05$); not significant when interaction is deleted; refer to Appendix Table O-1 for a detailed description of this interaction.

** (NS*)/** (ns*): Log₂ (current dioxin)-by-time-by-covariate interaction ($0.01 < p \leq 0.05$); marginally significant when interaction is deleted; refer to Appendix Table O-1 for a detailed description of this interaction.

** (...): Log₂ (current dioxin)-by-time-by-covariate interaction ($0.01 < p \leq 0.05$); significant when interaction is deleted; refer to Appendix Table O-1 for a detailed description of this interaction.

****: Log₂ (current dioxin)-by-time-by-covariate interaction ($p \leq 0.01$); refer to Appendix Table O-1 for a detailed description of this interaction.

TABLE 16-27. (Continued)
Summary of Current Dioxin and Time Analyses for Immunology Variables
Based on Minimal and Maximal Assumptions
(Ranch Hands Only)

Notes: P-value given if $p \leq 0.05$.

C*T: Log_2 (current dioxin)-by-time interaction hypothesis test.

≤ 18.6 : Log_2 (current dioxin) hypothesis test for Ranch Hands with time since end of tour of 18.6 years or less.

> 18.6 : Log_2 (current dioxin) hypothesis test for Ranch Hands with time since end of tour greater than 18.6 years.

A capital "NS" denotes relative risk/slope for ≤ 18.6 category less than relative risk/slope for > 18.6 category, relative risk 1.00 or greater for discrete analysis, or slope nonnegative for continuous analysis; a lowercase "ns" denotes slope for ≤ 18.6 category greater than slope for > 18.6 category, relative risk less than 1.00 for discrete analysis, or slope negative for continuous analysis.

TABLE 16-28.

**Summary of Categorized Current Dioxin Analyses for
Immunology Variables
(Ranch Hands and Comparisons)**

Variable	Unadjusted			
	All Categories	Unknown versus Background	Low versus Background	High versus Background
Physical Examination				
Composite Skin Test Diagnosis (D)	NS	NS	NS	NS
Laboratory				
CD2 Cells (C)	NS	ns	NS	NS
CD4 Cells (C)	NS	ns	NS	NS
CD8 Cells (C)	NS	NS	ns	NS
CD20 Cells (C)	NS	NS	NS	NS*
CD14 Cells (C)	NS	ns	ns	ns
CD25 Cells (C)	NS	NS	ns	NS
HLA-DR Cells (C)	NS	NS	NS	NS
CD4/CD8 Ratio (C)	NS	ns*	NS	NS
TLC (C)	NS	ns	NS	NS
Unstimulated PHA Response (C)	NS	ns	NS	NS
PHA Net Response - Conc. 1 (C)	NS	NS	NS	NS
PHA Net Response - Conc. 2 (C)	0.042	ns	NS	+0.025
PHA Net Response - Conc. 3 (C)	NS	ns	NS	NS
PHA Net Response - Across Day and Concentration (C)	NS	ns	NS	NS*
Maximum PHA Net Response (C)	0.037	ns	NS	+0.022
Unstimulated MLC Response (C)	NS*	NS	NS	+0.011
MLC Net Response (C)	NS	NS	NS	ns
NKCA 50/1 Net Response (C)	NS	NS	ns	ns
NKCA 50/1 Percent Release (C)	NS	NS	ns*	ns
NKCI 50/1 Net Response (C)	NS	ns	NS	ns
NKCI 50/1 Percent Release (C)	NS	ns	NS	ns
IgA (C)	0.013	-0.003	ns	NS
IgG (C)	NS*	-0.008	ns	ns
IgM (C)	NS*	NS	ns*	NS

C: Continuous analysis.

D: Discrete analysis.

+: Difference in means nonnegative for continuous analysis.

-: Difference in means negative for continuous analysis.

NS/ns: Not significant ($p > 0.10$).NS*/ns*: Marginally significant ($0.05 < p \leq 0.10$).Note: P-value given if $p \leq 0.05$.

A capital "NS" denotes relative risk 1.00 or greater for discrete analysis or difference in means nonnegative for continuous analysis; a lowercase "ns" denotes difference in means negative for continuous analysis; a capital "NS" in the first column does not imply directionality.

TABLE 16-28. (Continued)

**Summary of Categorized Current Dioxin Analyses for
Immunology Variables
(Ranch Hands and Comparisons)**

Variable	All Categories	Unknown versus Background	Adjusted	
			Low versus Background	High versus Background
Physical Examination				
Composite Skin Test Diagnosis (D)	NS	NS	NS*	NS
Laboratory				
CD2 Cells (C)	**NS	**(ns)	**(NS)	**(NS)
CD4 Cells (C)	NS	ns	NS	NS
CD8 Cells (C)	NS	NS	ns	NS
CD20 Cells (C)	** (NS)	** (NS)	** (NS)	** (NS)
CD14 Cells (C)	NS	ns	ns	ns
CD25 Cells (C)	NS	NS	ns	NS
HLA-DR Cells (C)	****	****	****	****
CD4/CD8 Ratio (C)	NS	ns*	NS	NS
TLC (C)	****	****	****	****
Unstimulated PHA Response (C)	NS	ns	NS	NS
PHA Net Response - Conc. 1 (C)	NS	NS	NS	NS
PHA Net Response - Conc. 2 (C)	NS	ns	NS	NS*
PHA Net Response - Conc. 3 (C)	****	****	****	****
PHA Net Response - Across Day and Concentration (C)	NS	NS	NS	NS
Maximum PHA Net Response (C)	NS	ns	NS	NS*
Unstimulated MLC Response (C)	NS	NS	NS	+0.038
MLC Net Response (C)	NS	NS	NS	ns
NKCA 50/1 Net Response (C)	NS	NS	ns	ns
NKCA 50/1 Percent Release (C)	NS	NS	ns	ns
NKCI 50/1 Net Response (C)	****	****	****	****
NKCI 50/1 Percent Release (C)	** (NS)	** (ns)	** (ns)	** (ns)
IgA (C)	0.007	-0.003	ns	NS
IgG (C)	NS	-0.028	NS	NS
IgM (C)	NS*	NS	ns*	NS

C: Continuous analysis.

D: Discrete analysis.

+: Difference in means nonnegative for continuous analysis.

-: Difference in means negative for continuous analysis.

NS/ns: Not significant ($p > 0.10$).NS*/ns*: Marginally significant ($0.05 < p \leq 0.10$).

** (NS)/** (ns): Categorized current dioxin-by-covariate interaction ($0.01 < p \leq 0.05$); not significant when interaction is deleted; refer to Appendix Table O-1 for a detailed description of this interaction.

****: \log_2 (current dioxin)-by-time-by-covariate interaction ($p \leq 0.01$); refer to Appendix Table O-1 for a detailed description of this interaction.

Note: P-value given if $p \leq 0.05$.

A capital "NS" denotes relative risk 1.00 or greater for discrete analysis or difference in means nonnegative for continuous analysis; a lowercase "ns" denotes difference in means negative for continuous analysis; a capital "NS" in the first column does not imply directionality.

TABLE 16-29.

Summary of Dioxin-by-Covariate Interactions from Adjusted Analyses of Immunology Variables

Variable	Assumption	Covariate
Model 1: Ranch Hands - Log₂ (Initial Dioxin)		
CD2 Cells	Minimal	ALC
CD8 Cells	Minimal	DRKYR
CD8 Cells	Maximal	ALC
CD20 Cells	Minimal	AGE
CD14 Cells	Minimal	ALC,PACKYR
CD25 Cells	Minimal	CSMOK,PACKYR,DRKYR
CD25 Cells	Maximal	CSMOK,PACKYR,DRKYR
HLA-DR Cells	Minimal	AGE
HLA-DR Cells	Maximal	AGE,ALC
TLC	Minimal	ALC
PHA Net Response - Conc. 1	Minimal	DRKYR
PHA Net Response - Conc. 1	Maximal	PACKYR
PHA Net Response - Conc. 3	Maximal	ALC
PHA Net Response [†]	Minimal	PACKYR
NKCA 50/1 Percent Release	Minimal	CSMOK,DRKYR
IgG	Minimal	DRKYR
IgM	Minimal	ALC
Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time		
Composite Skin Test Diagnosis	Minimal	AGE
CD4 Cells	Minimal	DRKYR
CD4 Cells	Maximal	AGE
CD14 Cells	Maximal	PACKYR
PHA Net Response [†]	Minimal	DRKYR
Unstimulated MLC	Minimal	DRKYR
Unstimulated MLC	Maximal	PACKYR
NKCI 50/1 Net Response	Minimal	RACE
NKCI 50/1 Net Response	Maximal	RACE
NKCI 50/1 Percent Release	Minimal	RACE
NKCI 50/1 Percent Release	Maximal	RACE
IgA	Minimal	CSMOK
IgA	Maximal	CSMOK
Model 3: Ranch Hands and Comparisons by Current Dioxin Category		
CD2 Cells	--	AGE,DRKYR
CD20 Cells	--	AGE
HLA-DR Cells	--	AGE
TLC	--	AGE,DRKYR
PHA Net Response - Conc. 3	--	ALC
NKCI 50/1 Net Response	--	RACE,ALC
NKCI 50/1 Percent Release	--	RACE,ALC,DRKYR

[†]Across mitogen harvest day and mitogen concentration.

interaction for all cell surface marker variables except CD4 and the CD4/CD8 ratio. The adjusted analyses under the maximal assumption generally were nonsignificant. The CD8, CD25, and HLA-DR cells exhibited significant initial dioxin-by-covariate interactions under this assumption. Similar to the unadjusted analysis, the adjusted analysis under the maximal assumption indicated there was a marginally significant positive association between the CD4/CD8 ratio and initial dioxin.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In general, the unadjusted analysis of the cell surface marker variables under both the minimal and maximal assumptions did not exhibit significant associations with current dioxin and time since tour (see Table 16-27). Under the minimal assumption, the unadjusted analysis of the nonzero CD25 cells displayed a significant negative association with current dioxin ($p=0.028$) for Ranch Hands with later tours (i.e., ≤ 18.6 years) and a nonsignificant positive association with current dioxin for Ranch Hands with early tours (i.e., >18.6 years). The two time strata associations cited above were found to differ marginally from each other. Under the maximal assumption, there were isolated marginally significant positive associations (i.e., for CD20 cells within time over 18.6 years, and CD4/CD8 ratio within time of 18.6 years or less).

For the cell surface marker variables, the adjusted analysis of the nonzero CD25 cells exhibited the same results under the minimal assumption as in the unadjusted analysis (i.e., a marginally significant current dioxin-by-time interaction, a significant negative association for Ranch Hands with late tours, and a nonsignificant positive association for Ranch Hands with early tours) because no covariates were retained in the adjusted model. For CD20 cells, Ranch Hands with an early tour under the minimal assumption exhibited a significant positive association ($p=0.043$). The adjusted analysis of CD4 cells displayed a significant interaction among current dioxin, time, and lifetime alcohol history under the minimal assumption and a significant interaction among current dioxin, time, and age under the maximal assumption. The adjusted analysis of CD14 cells under the maximal assumption exhibited a significant interaction among current dioxin, time, and lifetime cigarette smoking history.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

In the unadjusted analysis of each of the cell surface marker variables, the overall contrast for Ranch Hands in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category was nonsignificant (see Table 16-28). In the unadjusted analysis, the CD4/CD8 ratio exhibited a marginally significant difference only for the unknown versus background contrast (the Ranch Hands had the lower CD4/CD8 average) and the unadjusted analysis of CD20 had a marginally significant difference for the high versus background contrast (the Ranch Hands had the higher CD20 average).

In the adjusted analysis of CD2 cells, there were significant interactions between categorized current dioxin and age and between categorized current dioxin and lifetime alcohol history. An additional adjusted model without these interactions displayed a nonsignificant overall contrast for CD2 cells. For CD20 cells, the adjusted analysis contained a significant interaction between categorized current dioxin and age. A followup model without the interaction exhibited a nonsignificant overall contrast as well as nonsignificant Ranch Hand versus Comparison contrasts. The adjusted analysis of HLA-DR cells contained

a significant interaction between categorized current dioxin and age. The adjusted analyses of the other cell surface marker variables were nonsignificant.

Longitudinal Analysis of CD4/CD8 Ratio

For the most part, the longitudinal analyses of the CD4/CD8 ratio showed no significant differences. For the maximal analysis using current dioxin and time, Ranch Hands with more than 18.6 years since their tour displayed a significant increasing association between current dioxin and the change in the CD4/CD8 ratio (1987 ratio relative to the 1985 ratio). This change is opposite to that expected if dioxin caused immunodeficiency.

Laboratory Examination Variables: Quantitative Studies—TLC

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of TLC, both the minimal and maximal cohorts displayed a nonsignificant association between TLC and initial dioxin. Under the minimal assumption, the adjusted analysis contained a significant interaction between current alcohol use and initial dioxin. Without that interaction in the adjusted model, the association with initial dioxin was nonsignificant. Under the maximal assumption, the adjusted analysis also indicated that the relationship between TLC and initial dioxin was nonsignificant.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

Under both the minimal and maximal assumptions, the unadjusted analysis indicated that the current dioxin-by-time since tour interaction was nonsignificant for TLC. Also, the adjusted analysis of TLC based on the maximal assumption was nonsignificant for an interaction between current dioxin and time. Under the minimal assumption, the adjusted analysis of TLC exhibited a marginally significant interaction of current dioxin and time for the nonsignificant associations of the two time strata.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted analysis indicated that there were nonsignificant differences for TLC among the Ranch Hands in the unknown, low, and high current dioxin categories and Comparisons having background current dioxin category. The adjusted analysis of TLC for categorized current dioxin contained significant interactions between categorized current dioxin and age and between categorized current dioxin and lifetime alcohol history.

Laboratory Examination Variables: Functional Stimulation Tests

As part of the battery of functional stimulation studies, the following variables were analyzed: unstimulated PHA responses for harvest days 1 and 2 concurrently; an overall simultaneous analysis of six PHA net responses (PHA net response determined for each of 2 mitogen harvest days at 3 mitogen concentrations); the maximum of the six PHA net responses over mitogen harvest day and mitogen concentration; unstimulated MLC response; MLC net response; NKCA 50/1 net response; NKCA 50/1 percent release; NKCI 50/1 net response; and NKCI 50/1 percent release.

PHA Response

The expected effect of immunodeficiency on the PHA response is a reduction of cpm values due to impaired lymphocyte proliferation and less incorporation of radioactive precursor nucleotides into newly synthesized DNA. Furthermore, partial immunodeficiency would be reflected by an increase in the PHA concentration at which maximal response is stimulated (i.e., increase in minimal threshold for response).

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

Under both assumptions, the unadjusted and adjusted analysis of unstimulated PHA response exhibited nonsignificant associations with initial dioxin.

For the analysis of PHA considering 2 mitogen harvest days and 3 mitogen concentrations as repeated measure factors, there were significant interactions between initial dioxin and mitogen concentration under both assumptions. Consequently, unadjusted and adjusted analyses were performed at each mitogen concentration.

For mitogen concentration 1, the unadjusted analyses for both cohorts were nonsignificant. For the adjusted analyses of PHA at mitogen concentration 1, the minimal analysis contained a significant interaction between initial dioxin and lifetime alcohol history and the maximal analysis displayed a significant initial dioxin-by-lifetime cigarette smoking history interaction.

For mitogen concentration 2, the unadjusted analyses of PHA exhibited significant positive associations with initial dioxin under both the minimal and maximal assumptions ($p=0.016$ and $p=0.008$, respectively) and marginally significant positive associations with initial dioxin under both assumptions in the adjusted analyses. A negative response would be expected in impaired immunity.

For mitogen concentration 3, the unadjusted analyses of PHA net response contained nonsignificant associations with initial dioxin under both assumptions. The adjusted analysis of PHA net response for the minimal assumption was nonsignificant for an association with initial dioxin. Under the maximal assumption, the adjusted analysis exhibited a significant interaction between initial dioxin and current alcohol use.

Because the minimal cohort had an initial dioxin-by-harvest day interaction with a p -value between 0.01 and 0.05, unadjusted and adjusted analyses were also performed on the six PHA net responses across mitogen harvest day and mitogen concentration. The unadjusted analysis under the minimal assumption had a marginally significant positive association with initial dioxin and the adjusted analysis contained a significant interaction between initial dioxin and lifetime cigarette smoking history. An unadjusted analysis of PHA net response across day and concentration under the maximal assumption was nonsignificant.

For the unadjusted analyses under both assumptions, the maximum PHA net response displayed a significant positive association with initial dioxin ($p=0.005$ and $p=0.009$ for the minimal and maximal assumptions). For the adjusted analyses of the maximum PHA net response, both cohorts exhibited marginally significant positive associations.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

For the unadjusted and adjusted analysis of unstimulated PHA response, the interaction of current dioxin-by-time since tour was nonsignificant under both assumptions.

In the unadjusted analysis of the six PHA net responses under the minimal assumption, there was a marginally significant interaction between current dioxin and time. For time less than or equal to 18.6 years, there was a significant positive association between PHA net response and current dioxin ($p=0.006$) and a nonsignificant positive association for the other time stratum. Under the maximal assumption, the unadjusted analysis displayed a nonsignificant interaction between current dioxin and time. In the adjusted analysis of PHA net response under the minimal assumption, there was a significant interaction among current dioxin, time since tour, and lifetime alcohol history. A secondary analysis performed without the interaction exhibited a marginally significant current dioxin-by-time interaction. For time less than or equal to 18.6 years, there was a significant positive association ($p=0.033$) between PHA net response and current dioxin, and for time greater than 18.6 years a nonsignificant negative association. In the adjusted analysis under the maximal assumption, the interaction of current dioxin and time was nonsignificant.

In the unadjusted analysis of the maximum PHA net response under the minimal assumption, the interaction of current dioxin and time was nonsignificant. However, there was a significant positive association with current dioxin ($p=0.002$) for time less than or equal to 18.6 years. In the unadjusted analysis under the maximal assumption, there were positive associations with current dioxin for both time strata (time \leq 18.6, $p=0.049$ and time $>$ 18.6, $p=0.008$) but the interaction of current dioxin and time was nonsignificant. In the adjusted analysis of the maximum PHA net response under the minimal assumption, the interaction of current dioxin and time was nonsignificant but there was a significant positive association ($p=0.013$) with current dioxin for time less than or equal to 18.6 years. Under the maximal assumption, the adjusted analysis also displayed a nonsignificant interaction between current dioxin and time since tour; however, there was a marginally significant positive association for time over 18.6 years.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The unadjusted and adjusted analyses indicated that there were nonsignificant differences for unstimulated PHA response among the Ranch Hands in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category.

As a result of an interaction between categorized current dioxin and mitogen concentration, the unadjusted and adjusted analyses of PHA net response were performed separately at each mitogen concentration. For mitogen concentration 1, the unadjusted and adjusted analyses contrasting Ranch Hands in the unknown, low, and high current dioxin categories versus the Comparisons were nonsignificant. For mitogen concentration 2, the unadjusted analysis of the PHA net response contained a significant overall contrast ($p=0.042$) that was accompanied by a significant high versus background contrast ($p=0.025$). The adjusted analysis of the PHA net response at mitogen concentration 2 exhibited a nonsignificant overall contrast and a marginally significant contrast for the high versus background categories. For the two high versus background contrasts, Ranch Hands exceeded Comparisons on PHA net response. The unadjusted analysis of PHA net response

at mitogen concentration 3 was nonsignificant. The adjusted analysis contained a significant interaction with current alcohol use.

Unadjusted and adjusted analyses were performed without the interaction involving mitogen concentration (i.e., across mitogen harvest day and mitogen concentration). In the unadjusted analysis, the overall contrast was nonsignificant, but the high versus background contrast was marginally significant with Ranch Hands exhibiting a higher response than Comparisons. The adjusted analysis contained a nonsignificant overall contrast.

In the unadjusted analysis of the maximum PHA net response, the overall contrast was significant ($p=0.037$) and the high versus background contrast was also significant ($p=0.022$) with Ranch Hands being higher than Comparisons. The adjusted analysis contained a nonsignificant overall contrast and a marginally significant high versus background contrast with the Ranch Hand response again exceeding that of the Comparisons.

MLC

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

For unstimulated MLC response, the unadjusted analysis under the minimal assumption exhibited a nonsignificant association with initial dioxin. Under the maximal assumption there was a marginally significant positive association. The adjusted analysis for both assumptions exhibited a nonsignificant association between unstimulated MLC and initial dioxin.

For MLC net response, the unadjusted and the adjusted analyses under both assumptions were nonsignificant for an association with initial dioxin.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

In the unadjusted analysis of unstimulated MLC under the minimal and maximal assumptions, the interactions of current dioxin and time since tour were nonsignificant. However, there was a marginally significant positive association between unstimulated MLC and current dioxin for time over 18.6 years under the maximal assumption. The adjusted analysis contained a significant interaction among current dioxin, time, and lifetime alcohol history for the minimal cohort. The adjusted analysis of unstimulated MLC for the maximal cohort contained a significant interaction among current dioxin, time, and lifetime cigarette smoking history. Followup analyses without the interaction were nonsignificant for the maximal cohort.

For MLC net response, the unadjusted and the adjusted analyses of both assumptions contained nonsignificant interactions between current dioxin and time since tour.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

In the unadjusted analysis of unstimulated MLC response, the overall contrast of Ranch Hands in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category was marginally nonsignificant. The high versus background contrast of unstimulated MLC response was significant ($p=0.011$), with Ranch

Hands in the high current dioxin category having a higher unstimulated MLC response than Comparisons in the background current dioxin category. In the adjusted analysis of unstimulated MLC response, the overall contrast was nonsignificant but the contrast for high versus background was significant ($p=0.038$) with the Ranch Hands in the high current dioxin category being greater on unstimulated MLC than the Comparisons.

For both the unadjusted and the adjusted analyses of MLC net response, the overall contrast of Ranch Hands in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category were nonsignificant.

Natural Killer Cell

Both stimulated and Interleukin 2 stimulated natural killer cells decline with progression of malignancies and show only a fraction of normal activity with advanced disease. They are probably most important early in carcinogenesis to screen for and destroy tumor cells when their numbers are still small.

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

The unadjusted and adjusted analyses displayed nonsignificant associations between NKCA 50/1 net response and initial dioxin under both assumptions.

Similarly, under both assumptions, the unadjusted analysis of NKCA 50/1 percent release was nonsignificant for an association with initial dioxin. Under the minimal assumption, the adjusted analysis of NKCA 50/1 percent release contained significant interactions between initial dioxin and current cigarette smoking and between initial dioxin and lifetime alcohol history. Under the maximal assumption, the adjusted analysis of NKCA 50/1 percent release was nonsignificant.

Under both the minimal and maximal assumptions, the unadjusted and the adjusted analyses exhibited nonsignificant associations between NKCI 50/1 net response and initial dioxin, as well as NKCI 50/1 percent release and initial dioxin.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

For NKCA 50/1 net response and NKCA 50/1 percent release, the unadjusted analysis under both assumptions exhibited nonsignificant interactions between current dioxin and time since tour. Under the minimal assumption, the adjusted analyses of NKCA 50/1 net response and NKCA 50/1 percent release also contained nonsignificant interactions between current dioxin and time since tour. Under the maximal assumption, the adjusted analysis of NKCA 50/1 net response exhibited a marginally significant current dioxin-by-time interaction. For time less than or equal to 18.6 years, there was a nonsignificant positive association between NKCA 50/1 net response and current dioxin. For time greater than 18.6 years, there was a marginally significant negative association between NKCA 50/1 net response and current dioxin. The adjusted analysis of NKCA 50/1 percent release displayed a nonsignificant current dioxin-by-time interaction under the maximal assumption.

Under the minimal assumption, the unadjusted analysis of NKCI 50/1 net response and NKCI 50/1 percent release displayed nonsignificant interactions between current dioxin and

time since tour. Under the maximal assumption, the unadjusted analysis of NKCI 50/1 net response and percent release displayed marginally significant current dioxin-by-time interactions. For time less than or equal to 18.6 years, there were marginally significant positive associations with current dioxin for both NKCI 50/1 variables. For time greater than 18.6 years, there were nonsignificant negative associations with current dioxin for both NKCI 50/1 net response and percent release.

Under the minimal assumption, the adjusted analyses of NKCI 50/1 net response and NKCI 50/1 percent release both contained a significant interaction among current dioxin, time, and race. Followup analyses without the interactions in the adjusted models displayed marginally significant interactions between current dioxin and time since tour. Under the minimal assumption, both NKCI 50/1 variables displayed a significant positive association with current dioxin for time less than or equal to 18.6 years and a nonsignificant negative association for time over 18.6 years. The adjusted analyses of NKCI 50/1 net response and NKCI 50/1 percent release under the maximal assumption also displayed significant interactions among current dioxin, time since tour, and race.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the unadjusted analyses of NKCA 50/1 net response and percent release and NKCI 50/1 net response and percent release, the overall contrasts of Ranch Hands in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category were nonsignificant. Except for a marginally significant low versus background contrast on NKCA 50/1 percent release, the individual Ranch Hand versus Comparison contrasts were nonsignificant for these unadjusted analyses.

In the adjusted analysis of NKCA 50/1 net response and NKCA 50/1 percent release, the overall contrasts of Ranch Hand in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category were nonsignificant. The adjusted analysis of NKCI 50/1 net response contained significant interactions between categorized current dioxin and race and between categorized current dioxin and current alcohol use. In the adjusted analysis of NKCI 50/1 percent release, there were significant interactions between categorized current dioxin and each of the following covariates: race, current alcohol use, and lifetime alcohol history. Because the p-values of each of the three interactions exceeded 0.01 but were less than 0.05, a followup model without the three interactions was investigated. The overall contrast for that model was nonsignificant.

Quantitative Immunoglobulins

Serum immunoglobulins may show either increases or decreases related to immunodeficiencies or malignancy. Severe defects of B cells can result in near absence of IgG, IgA, and IgM (hypogammaglobulinemia) or selective deficiency of one or two of these immunoglobulin classes. Hypogammaglobulinemia is frequently (but not always) a consequence of lymphatic cancer due to the replacement of normal immunoglobulin secreting cells with malignant ones. In some B-cell disorders, there is proliferation of a single (malignant) clone of cells that inappropriately synthesize a monoclonal immunoglobulin (IgG, IgA, IgM, IgD, or IgE, with only kappa or lambda light-chain type) (e.g., multiple myeloma, Waldenstrom's macroglobulinemia). In other disorders involving T cells such as AIDS, there is frequently a polyclonal increase of all serum immunoglobulins due to impaired (down) regulation of their synthesis. Liver disease and especially cirrhosis are also well known to

cause polyclonal increases of serum immunoglobulins due to impaired down-regulation. Chronic infections (which may be due to impaired immunity) also lead to polyclonal increases probably due to long-term stimulation with microbial antigens.

Model 1: Ranch Hands - Log₂ (Initial Dioxin)

In the unadjusted analysis of IgA under the minimal assumption, the association with initial dioxin was nonsignificant. However, the unadjusted analysis of IgA under the maximal assumption contained a significant positive association with initial dioxin ($p=0.009$). In the adjusted analysis of IgA, there was a significant positive association with initial dioxin under the minimal assumption ($p=0.019$) and also under the maximal assumption ($p=0.003$).

For both the unadjusted analysis of IgG and IgM, the association with initial dioxin was nonsignificant under the minimal and maximal assumptions. Under the minimal assumption, the adjusted analysis of IgG contained a significant initial dioxin-by-lifetime alcohol history and the adjusted analysis of IgM contained a significant interaction between initial dioxin and current alcohol use. Followup models without these interactions displayed nonsignificant associations with initial dioxin. Under the maximal assumption, the adjusted analysis of IgG and IgM displayed nonsignificant associations with initial dioxin.

Model 2: Ranch Hands - Log₂ (Current Dioxin) and Time

For the unadjusted analyses of IgA, IgG, and IgM under the minimal assumption, the interaction of current dioxin and time since tour was nonsignificant. Under the maximal assumption, the current dioxin-by-time interaction for each of the three immunoglobulins was also nonsignificant. However, a marginally significant positive association between IgA and current dioxin was found for time over 18.6 years, and a marginally significant negative association between IgM and current dioxin for time less than or equal to 18.6 years was also found.

In the adjusted analysis of IgA under the minimal assumption, there was a significant interaction among current dioxin, time since tour, and current cigarette smoking. A followup model without that interaction exhibited a nonsignificant interaction between current dioxin and time and a marginally significant positive association with current dioxin for time less than or equal to 18.6 years. In the adjusted analysis of IgA under the maximal assumption, there was also a significant interaction among current dioxin, time, and current cigarette smoking. A followup model without the interaction displayed a nonsignificant interaction between current dioxin and time but a significant positive association ($p=0.027$) with current dioxin was found for time over 18.6 years. Under both the minimal and maximal assumptions, the adjusted analysis of IgG and IgM displayed nonsignificant interactions between current dioxin and time. In the adjusted analysis of IgM under the maximal assumption, there was a significant negative association with current dioxin for time less than or equal to 18.6 years ($p=0.027$).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

In the unadjusted analysis of IgA, the overall contrast was significant ($p=0.013$) for Ranch Hands in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category. The unknown versus background contrast was significant ($p=0.003$) with the Comparisons having the larger IgA mean value. In the

unadjusted analysis of IgG, the overall contrast was marginally significant and the unknown versus background contrast was again significant ($p=0.008$) with Comparisons having the larger IgG mean. The unadjusted analysis of IgM displayed a marginally significant overall contrast and a marginally significant low versus background contrast with Comparisons having the larger IgM mean.

In the adjusted analysis of IgA, the overall contrast of Ranch Hands in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category was significant ($p=0.007$). For IgA, the unknown versus background contrast was also significant ($p=0.003$) with the Comparisons having the larger adjusted IgA mean. In the adjusted analysis of IgG, the overall contrast of the current dioxin categories Ranch Hands and Comparisons was nonsignificant, but the unknown versus background contrast was significant ($p=0.028$) with the Comparisons having the larger adjusted IgG mean. In the adjusted analysis of IgM, the overall contrast was marginally significant and the low versus background contrast was also marginally significant, with the Comparisons having the larger adjusted IgM mean.

CONCLUSION

In general, the composite skin test diagnosis results were not associated with serum dioxin levels. The Ranch Hand analyses using initial dioxin, and the analyses using current dioxin and time since tour, generally displayed nonsignificant relative risks less than 1. For the analyses contrasting Ranch Hands with unknown, low, and high current dioxin to Comparisons with background current dioxin, the estimated relative risks were greater than 1 but nonsignificant.

For the most part, the cell surface marker variables and total lymphocyte count did not display significant associations with serum dioxin. The longitudinal analyses of the CD4/CD8 ratio did not consistently show significant differences in the 1987 ratio relative to the 1985 measurement of the ratio.

For the analyses of PHA net responses, significant or marginally significant positive associations with initial dioxin were found for the PHA net responses obtained for mitogen harvest concentration 2 and for the maximum of the six PHA net response from 3 mitogen concentrations and 2 mitogen harvest days. For the analyses involving current dioxin and time since tour, the maximum PHA net response also displayed some significant or marginally significant positive associations. The analyses contrasting Ranch Hands and Comparisons indicated that the Ranch Hands with high current dioxin had, on average, significantly higher or marginally higher maximum PHA net responses than the Comparisons. A similar pattern involving Ranch Hands in the high current dioxin category and Comparisons was also found for the analysis of PHA net responses at mitogen concentration 2. Depressed immune function would be expected to demonstrate lower PHA net response.

For unstimulated MLC and MLC net response, the three analysis approaches generally displayed nonsignificant associations with serum dioxin. For the analysis involving Ranch Hands in the high current dioxin category and Comparisons in the background current dioxin category, the Ranch Hands had a significantly higher unstimulated MLC mean.

The analyses of the NKCI 50/1 net response and NKCI 50/1 percent release variables were, for the most part, nonsignificant. The adjusted analyses based on current dioxin and time since tour, and the analyses using Ranch Hands and Comparisons, contained significant interactions with race. For both variables, the non-Black stratum had significant current dioxin-by-time interactions with significant positive associations for time less than or equal to 18.6 years and nonsignificant negative associations for time over 18.6 years. In the analyses of the 1987 followup examination data, significant interactions with race were also found in which Black Comparisons had the lowest mean response.

For the most part, significant positive associations were found between IgA and initial dioxin and nonsignificant positive associations were found for IgG and IgM. A negative association would be expected in immunologic deficiency. The analyses for IgA, IgG, and IgM using current dioxin and time since tour were, for the most part, nonsignificant. For the three immunoglobulins analyzed, the overall contrasts of Ranch Hands in the unknown, low, and high current dioxin categories versus Comparisons in the background current dioxin category were generally significant or marginally significant. For IgA and IgG, the contrasts of Ranch Hands in the unknown current dioxin category versus Comparisons in the background current dioxin category were significant with the Ranch Hands having the lower immunoglobulin averages. For IgM, the contrasts of Ranch Hands in the low current dioxin category versus Comparisons in the background current dioxin category were marginally significant with the Ranch Hands again having the lower averages. Ranch Hands in the higher category were not significantly different from the Comparisons, an observation that is not supportive of a dose-response effect.

In summary for the immunologic assessment, the maximum PHA net response and the PHA net response at mitogen concentration 2 displayed significant positive associations with serum dioxin, an observation opposite to expectations if dioxin exerted a depressant effect on immune function. Although the composite skin test diagnosis displayed a significant group difference in the 1987 examination report, no significant associations with serum dioxin were found. Also in the report on the analysis of the 1987 examination data, the covariate of race was involved in group-by-covariate interactions for the natural killer cell assay variables. The clinical relevance of such race interactions was not interpretable. For the serum dioxin analyses, race was also found to be involved in dioxin-by-race interactions for net response and percent release of the natural killer cell assays with Interleukin 2, but its clinical relevance could not be determined since the numbers of Blacks in the analyses were too small.

The indices of immune responses analyzed in this chapter provided a comprehensive reflection of in vivo and in vitro immune function in the study population. No clinically significant indicators reflecting a relationship between the current body burden of dioxin or the extrapolated initial exposure and immune function were found. The increased IgA levels could represent a chronic inflammatory response to dioxin exposure, as do elevated erythrocyte sedimentation rates (in the general health assessment) and white blood cell and platelet counts (in the hematologic assessment).

CHAPTER 16

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